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The Effects of Estrogen on the Mammary Gland of Mice Injected During Pregnancy, Lactation, and Retrogression*

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Transformation of normal mammary gland cells of mice into cancer cells is preceded by a period of noncancerous growth, which, step by step, may lead to the end stage of cancerous growth. A similar process occurs in vagina and cervix. This cancerous change takes place after a long-continued stimulation of the tissue by hormones which causes an intensification of growth processes, gradually exceeding normal limits. In the end, the growth process becomes abnormal and enters the cancerous stage (7-13). However, there is another interpretation of the cancerous transformation which assumes that it is caused by changes in chromosomes and genes in somatic cells, by a somatic mutation. Such a mutation would be a random occurrence, found more often the larger the number of cells observed.

During pregnancy there is a rapid multiplication of mammary gland cells with the result that a small amount of formerly resting tissue is changed into a very extensive organ. Following labor, this is transformed into a lactating organ and retrogresses when lactation is completed. It seemed of interest to test the effect of large amounts of estrogen[1] on this enlarged and active organ to determine whether the hormone was able to induce cancerization of such tissue more readily than mammary gland tissue in nonpregnant and nonlactating mice. Furthermore, it might be expected that such investigations would help to explain, perhaps, the greater incidence of mammary gland carcinoma in breeding mice as compared with virgin mice. For these experiments we selected mice of strain A, in which the incidence of mammary gland carcinoma in breeding mice is relatively high, and mice of strain CBA, in which the incidence is relatively low. We also compared the retrogressive changes normally following pregnancy and lactation with those which occur under the influence of large amounts of estrogen injected subcutaneously.

Two series of experiments were carried out. In the first, injections were begun 2 to 10 days before the end of pregnancy and continued afterwards for 2 weeks, 1, 2, or 3 months. Some of these mice nursed their young, while others killed them soon after birth. In the second series, injections were begun from 3 to 5 days following labor and were continued for the same length of time as in the first series. In each case injections of 100 rat units of estrogen dissolved in oil were given subcutaneously twice weekly. All of the mice had been breeding but the number of litters they had nursed differed in different cases. Mice of both strains, A and CBA, were treated in this way. The age of these mice varied in most instances between 6 and 8 months, the lowest age being 5.5 months and the highest about 10 months. As a rule, 2 A mice and 2 CBA mice were used for each of the periods tested. Two parallel series of experiments were made. In series A, the injections began before the end of pregnancy while in series B they began several days after the young had been born. The time during which the mother nursed her young varied. In a considerable number of instances the mother killed her young a short time after the beginning of the injections. The mammary glands and the ovaries of all of these mice were examined microscopically, the latter organ in serial sections. Altogether, 14 control and 32 injected mice, divided equally between strain A and strain CBA, were used.

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[1] We are indebted to Dr. Erwin Schwenk of the Schering Corporation for supplying us with Progynon-B, the estrogenic substance used in these experiments.—AUTHORS.

For the sake of brevity, we shall describe only changes observed at certain periods following pregnancy and omit the description of those observed at other periods.

Normal Retrogression Following Pregnancy in Mice of Strain A and Strain CBA

Two weeks after labor a very marked retrogression had taken place in the mammary gland in mice which had been prevented from nursing their young after birth. The gland tissue then consisted of scattered atrophic ducts, with or without a few atrophic acini. The degree of atrophy differed in different structures. In some ducts it had progressed further than in others, which were of an intermediate character. Other ducts and acini were still lined with medium-sized epithelium. No mitoses were visible and only slight remnants of secretion were seen in certain acini or small ducts. The remaining small groups of acini were mostly composed of solid cells. In some large ducts shrinking had also occurred, and in the surrounding fibrous and hyaline tissue branching ducts likewise were shrunken and atrophy had taken place in the connective tissue cells enclosed in this material. It seemed that the fibrous tissue around these ducts had undergone softening in places. In the small vacuolated fat tissue the ducts and acini had, as usual, entirely disappeared or were in the process of disappearing. Occasionally the presence of dense hyaline tissue around the ducts contributed to their shrinkage.

After one month.—The mammary gland showed further marked retrogression; but in different animals the degree of retrogression and the condition of the ducts and acini varied somewhat, apparently without definite relation to whether the mice did or did not nurse their young. On the whole, the mammary glands consisted of atrophic ducts, with or without a small number of retrogressing acini. However, certain variations were observed. In one animal which had been nursing her young there were still several small groups of fairly well-preserved acini. In these, the lining epithelial cells were larger and secretion occurred in some cells, but the secretion vacuoles were usually very small. As a rule, there was no evidence of secretion. In different ducts and acini the degree of retrogression varied and there were structures intermediate between fully atrophic and the usual medium-sized ducts or acini. Mitoses were seen in small ducts or acini in only one mouse, and in this case the ducts and acini were, on the whole, better developed.

After two months.—Atrophy in ducts and acini had progressed, the mammary gland consisting largely of very atrophic ducts surrounded by a few atrophic acini. Hyaline connective tissue, which was found around some ducts, usually accentuated the atrophy. In some places a few small collections of acini with slight secretion were seen, and in one place there were some better developed ducts and acini composed of solid cells. The furthest advanced processes were observed in a somewhat larger group of acini which possessed good lumens and were lined with high cells in which many secretion vacuoles were seen. At other points, in some acini surrounding the ducts, a few small vacuoles had been produced and in certain of these acini the nuclei were enlarged. But these were isolated changes. On the whole, a marked reduction in size and number of ducts and acini was characteristic of these glands and secreting acini might be absent entirely over wide areas.

After three months.—Conditions were similar to those seen after 2 months. There were scattered and atrophic ducts surrounded by a few isolated acini. Occasionally, a few better developed acini were seen in which some of the cells showed secretion vacuoles. Formation of a small clump of atrophic or intermediate acini had also taken place. Very rarely, moderately large acini containing some secretion were seen, but in a few places groups of as many as 8 to 10 acini in a section, surrounded by fibrous tissue, appeared, and in a few of these cells mitoses were noted. The mode of regression in strain A and in strain CBA was the same.

I. Experiments in which Injections of Estrogen Were Continued for Two Weeks

Series A.—In cases in which the mother had nursed her young until near the time of examination there were groups of acini still visible, as many as 8 to 14 acini appearing in a group in a single section. Secretion was noted and was quite marked in some places, but mitoses were very rarely seen in the acinus cells. On the whole, much retrogression had taken place in the gland and completely atrophic ducts and acini were found, notwithstanding the injection of large amounts of estrogen. However, an atrophy affecting the whole mammary gland was not the usual result. There still were acini and groups of acini in an active state, while others were in a condition intermediate between the normal resting state and atrophy. In cases in which the nursing was interrupted at an early date during the period of injections, the number of acini composing groups around the ducts was smaller, perhaps 3 or 4 acini being seen in a section, and the amount of secretion was less, the majority of these groups being without signs of secretion. Some acini were fairly well developed and had solid cells. There were various transitions between these two types of mammary gland. Here also were some atrophic ducts and acini and the pressure of the

hyaline tissue surrounding the ducts increased the atrophy.

Series B.—Three of these mice nursed their young until, or almost until, the time when the experiment was terminated 14 days after the beginning of the injections, while the fourth mouse nursed her young for 7 days after delivery, the injections being continued for 12 days longer. In this last case, marked retrogression of the mammary gland had taken place during the period when nursing had been suspended. There was still much mammary gland tissue present, but the ducts were thin and there were only small collections of small, nonsecreting acini lined with small-sized cells. Some acini and ducts were entirely atrophic, but the majority were of an intermediate character and retrogression was not yet complete. In a few of the small acini some secretion vacuoles occurred, although rarely. In addition, some acini were lined with large cells possessing large nuclei, and there were also some large acini containing secretion vacuoles and secreted colloid material in the central cavity. In this animal, then, estrogen did not prevent the retrogression of the gland, but retarded it in comparison with the control glands and with the glands of mice in which the injections began during the last part of pregnancy.

In a second experiment, in which the young were still with the mother at the time when the experiment was completed, the typical picture of a lactating gland was found over wide areas. In another part the mammary gland had undergone retrogression and there were atrophic or intermediate ducts and acini. Some of the ducts were lined with good-sized epithelium in which secretion vacuoles were seen; also, some of the acini had secretion vacuoles. Transitions occurred between these two types of tissue.

In a third (A strain) mouse, 9 months old, a mammary gland tumor had developed at an age when spontaneous tumors begin to appear in the A strain. The gland consisted, in most places, of medium-sized or slightly smaller ducts with some small groups of acini, while in other areas there were merely atrophic ducts. There were still some small lobules and groups, showing 10 to 12 acini in a section, in which the cells were of good size and had large secretion vacuoles. Areas of typical lactating gland were no longer seen, these being in process of complete retrogression.

In a fourth mouse a similar condition was noted, the mammary gland likewise representing an intermediate state of retrogression from lactation. There were many small groups of acini, 8 to 14 in one section. Much secretion and large secretion vacuoles were noted. There were also solid cells, in which, however, no mitoses occurred, and in addition, some still larger groups of acini reaching the size of small lobules were seen. No fully atrophic ducts or acini were observed. In one place a precancerous area was found in this actively secreting gland tissue. In this area the epithelium of ducts and acini was very high and some of the nuclei of these cells possessed one large, centrally situated nucleolus. There were mitoses but not many secretion vacuoles in these hypertrophic cells. A gradual transition took place from this precancerous tissue to the small lobules of the adjoining mammary gland tissue.

In general, we may conclude that in this early stage following the end of pregnancy the injections of estrogen exerted a stimulating effect on the mammary gland which delayed retrogression without, however, preventing it. In one mouse the stimulation led to the formation of precancerous tissue in a single place. On the whole, the glands in which injections were begun several days after the young had been born showed more activity and less retrogression than the glands in which the injections were started towards the end of pregnancy. It is furthermore of interest that in the glands of control animals, as well as in those of the injected ones, we had to deal not with general effects extending equally over the whole tissue, but with great inequalities in the progress of the changes in different parts of the gland which led to secretion and growth processes in some areas and to marked retrogression and atrophy in others. Both of these processes went hand in hand in the same gland, but to an unequal degree in different mice.

II. Experiments in which Injections Were Given for Two Months

Series A.—Injections began 2 to 10 days before delivery. In one case the young were killed by the mother soon after delivery; in the other 3 cases nursing continued for a number of days, but in all of them the young were dead by the 10th day following delivery. In these mice retrogression of ducts and acini had progressed, as compared with the findings at the earlier periods. On the whole, the number of acini was much reduced and the large majority of those that were left were either atrophic or in an intermediate condition. In the mammary glands of each of these animals, there were some groups of better preserved acini in which some of the large cells contained secretion vacuoles; in other groups the cells were solid. In several animals these groups were small, while in others a few larger groups also occurred. Some collections of atrophic acini were surrounded by fibrous tissue which increased the atrophy, and hyaline fibrous tissue had a similar effect on ducts. Likewise, the change from large to small vacuolated fat tissue had the usual, very injurious effect on glandular structures.

A few mitoses were seen in solid cells in the mammary gland of only one of these mice.

Series B.—In 4 mice, injections of estrogen began 5 to 7 days after delivery. In the mammary gland of these animals, atrophy of ducts and acini predominated over well developed secreting or solid gland tissue, but to an unequal degree, the amount of active tissue varying in different mice. Groups of secreting acini of different sizes, up to the size of small lobules, were observed in some animals. In one mouse scattered atrophic and intermediate ducts and acini predominated, with only small groups of acini having good lumens and lined with medium-sized or larger cells showing large secretion vacuoles. In another mouse which had nursed her young for 2 weeks after the beginning of the injections and in which the injections had extended over a period of 2 months and 12 days, in addition to many atrophic ducts which were surrounded by a very small number of atrophic acini, much secreting tissue was found in which the acini had large secretion vacuoles and in which the secreted material filled the lumen of the ducts. In this mouse also, lobules occurred composed of secreting acini, situated in the periphery of the lobule, and of atrophic acini and ducts surrounded by hyaline fibrous tissue in the central portion. In another animal many nuclei in some active acini contained large central nucleoli. In this animal a mitosis was seen in a duct which was lined with solid cells and which connected with the secreting acini.

Discussion

From these and other unpublished experiments it may be concluded that large amounts of estrogen injected into mice at the end of pregnancy or during the early stages of lactation, and continued for varying lengths of time, do not prevent the retrogression of the mammary gland; nor do they lead, under these circumstances, to a direct carcinomatous transformation of the mammary gland. There was no relation between the amount of mammary gland produced, and thus no relation between the number of cells available for a somatic mutation, and the development of cancer. In one case, however, 2 weeks after the beginning of the injections of estrogen, a condition which may be considered as precancerous was observed, and in another case a marked effect, perhaps not yet to be classed as precancerous, was noted after injections had been given for 3 months. It is quite possible that injections continued for still longer periods might have led to the production of carcinoma in some animals. But the fact must be considered that the mice used were mostly as old as 6 to 8 months at the beginning of the experiment, and were still older at the end of the experiment. They were therefore approaching the age when spontaneous tumors develop in mice belonging to strains having high tumor rates. However, the direct change of the greatly enlarged mammary gland of pregnancy, or of the functionally active mammary gland of lactation, into cancerous tissue was interfered with by the retrogressive changes which occurred at the end of this period, notwithstanding the continued injection of very large doses of estrogen at regular intervals for varying periods of time. Estrogen did not prevent these retrogressive changes which closely resembled the corresponding changes occurring normally at the end of pregnancy and lactation. It was presumably the lack of placenta, or the corpus luteum of pregnancy, which was responsible for retrogression following pregnancy; and estrogen, which otherwise exerts a stimulating effect on the growth of the mammary gland and may cause its cancerous transformation under certain hereditarily transmitted constellations, was unable to take the place of the stimulating substance given off by one of these organs.

Yet, while estrogen could not prevent the retrogressive changes in these mammary glands, it did exert a definite stimulation on the mammary gland resulting both in growth and in secretory processes, which occurred in an intensified form in these artificially estrogenized mice as compared to normal control mice. Two processes, then, go hand in hand in the treated mice: (a) retrogressive changes in ducts and acini, and (b) initiation of growth and secretory processes. The retrogressive changes had not yet reached their maximum 2 weeks after the completion of pregnancy and they continued for some time afterwards. Gradually, growth and secretory processes increased during the course of the injections. The processes taking place in normal control and in experimentally estrogenized mice are, therefore, very similar. In normal mice, among the completely atrophic ducts and acini in a gland in which the large majority of acini have disappeared, there may also occur isolated acini or small groups of acini of a larger size, lined with solid or with secreting cells. In the solid cells, which are characterized by the lack of secretion vacuoles and by a consistent cytoplasm, mitoses may be found but they are rare. Likewise in the injected mice mitoses were not found frequently, although they were noted more often than in normal mice, in which they occurred presumably at the beginning of a sexual cyclical period. The processes taking place in normal mice and in experimentally estrogenized mice differ, then, in the amount of active gland tissue that is produced, this being greater in the injected mice. In both, estrogen is responsible for the glandular activity, but while this is initiated presumably by the estrogen normally produced in

the control mice, in the treated mice there is added the effect of large quantities of injected estrogen.

While there can be no doubt about the stimulating action of the estrogen experimentally administered or about the increase in active mammary gland tissue in the animals thus treated when compared with the controls, there are, in both series, considerable differences between individual mice which had been treated in the same way and examined at corresponding periods. The following statements are, therefore, only provisional and need to be tested further in subsequent investigations. It seems that 2 weeks, and perhaps also 1 month, after the beginning of the injections the mammary glands in mice of series B were better developed than in mice of series A, in which the administration of estrogen started during the last period of pregnancy. Furthermore, there was an indication that the changes in the mammary gland followed a definite course. Retrogressive changes in ducts and acini had apparently not yet reached their highest point 2 weeks after the beginning of the injections; they were more accentuated during the later periods. However, in the injected mice, and to a lesser extent also in the control mice, some ducts and acini regained their growth and secretory activity and thus variable amounts of active tissue developed. It appears that the amount of active gland tissue was, in general, greater after 3 months than after 1 month of observation; this applies especially to the injected animals, but perhaps also, although less clearly, to the control series. Besides, it seemed that in those animals which nursed their young for a longer time, the amount of active gland tissue was larger than in those in which the young died soon after birth; this was apparently the case especially if the animals were examined at the earlier periods. There was also some indication that a marked loss in body weight during the course of the experiment tended to diminish the secretory activity in the mammary gland.

If we compare the condition of the gland tissue found in the A and CBA strains of mice, there was no parallelism between the tendency to develop mammary gland carcinoma and the amount of active tissue found in the control and in the injected animals of these strains. There was at least as much active tissue present, on the average, in the CBA mice with a low cancer rate, as in the A mice, which possess a much higher cancer rate. This is true in both series A and series B. Indeed, the 2 mice examined after 2 weeks and 3 months, respectively, in which a localized area underwent changes which approached a precancerous condition, happened to belong to the CBA strain. In one case, more gland tissue or more secretion was found in an A mouse, while in another case, under otherwise similar conditions, more gland tissue occurred in a CBA mouse. Under these conditions we did not think it necessary to differentiate in our description between the mice belonging to these 2 strains. These findings are in agreement with the fact, indicated already by us, that different strains may differ in regard to the stage in which the cancerous changes set in. If we compare the CBA and A strains, we find that in CBA mice, injected with large doses of estrogen, the preparatory growth period may begin about as early as in A mice similarly treated; but in the former, the mammary gland develops well only up to the approach to the precancerous stage. This last stage is not usually reached in this strain, although the development of the gland tissue preceding this stage is about as active as in A mice. In the latter, the transition to the precancerous and cancerous stage takes place much more readily than in the CBA mice. In evaluating these findings we must take into consideration the following facts: (a) strain A mice, in our experience, differed in some essential respects from mice of strains D and C3H, which had the highest cancer incidence. As compared to the latter strains, the mammary gland of mice belonging to strain A showed a considerable resistance to growth stimulation. (b) The period during which the mice were exposed to the action of growth stimuli did not extend over 3 months, and (c) the time during which these mice were observed was characterized by the conditions peculiar to pregnancy, lactation, and retrogression, during which, as far as is known, no difference exists between the different strains. However, it might be of interest to compare the behavior of strains C3H or D with that of C57, strains which are much more divergent than strains A and CBA.

Experiments of Turner and Frank (14), and of Gardner, Diddle, Allen, Smith, and Strong (1-4), are of interest in connection with our observations. Turner and Frank noted that, in the rabbit, injection of estrogen causes a development of the mammary gland up to a certain point. When this point has been reached, retrogressive changes set in, even if the administration of estrogen is continued. Gardner and his collaborators found that when young mice belonging to strains with high incidence of tumors as well as to the CBA strain, were injected with small or larger doses of estrogen, nodules visible to the naked eye developed in the mammary gland in the course of the next few months. These nodules consisted of active mammary gland tissue similar to that found during pregnancy or lactation; they were situated in inactive and even in atrophic gland tissue. Similar nodules also developed spontaneously in female mice, but they increased in number with advancing age and persisted during the stage of retro-

gression following pregnancy and lactation. They were more frequent in strains with high incidence of tumors than in strains with the lowest incidence of tumors. These investigators suggested, therefore, that such localized nodules may be considered as the place of origin of mammary gland cancer. These findings, in common with those reported here and others reported previously (5, 6), show that localized growth and secretion processes may be associated elsewhere in the mammary gland with retrogressive changes.

In the experiments reported by us there was a definite relation between growth and secretion processes in the mammary gland tissue. As a rule, acini first undergo hypertrophy and only secondarily do secretion processes set in, as evidenced by the appearance of vacuoles in these cells. But such hypertrophic cells were also more prone to undergo mitotic proliferation, preceding the period of active secretion, than were cells of medium-sized, inactive acini. It was only in exceptional cases that secretion vacuoles were found in the smaller ducts and acini of medium-sized gland tissue, or even in slightly atrophic structures, and it is possible that the cells had actually been enlarged at the time when the secretion set in, but subsequently had undergone retrogression.

The dependence of secretory processes on a previous growth period noted in these experiments is in agreement with the observation that, as a rule, the administration of lactogenic hormone, in order to be effective, must be preceded by a growth of the mammary gland, taking place either spontaneously, which means under the influence of hormones given off by the ovary and, in some instances, by the placenta, or taking place experimentally in cases in which the hormones are administered to the animal. These observations correspond, furthermore, to the fact that the precancerous and cancerous growth usually occurs in tissue in which both hypertrophic growth and secretion processes are combined.

The focal character of the growth and secretory processes in the glands in control as well as in injected animals is contrary to what might be expected; it might be assumed that the stimulation by hormones would affect all parts of the gland in about the same manner. This focal response was not due to variations in the age of the mice. As stated, in the large majority of the animals it varied within the rather narrow range of 6 to 8 months; the lowest age was 5.5 months and the highest was 10 months. Furthermore, the time when lactation had ceased did not determine these focal variations. They were found quite generally without relation to the time when lactation had been interrupted in individual cases. Neither can these variations be due to differences in the stage of the estrous cycle at which examination took place, because they were a typical occurrence which cannot have depended upon peculiarities of this kind.

The mammary gland exhibits a homogeneous character during pregnancy and lactation, but under other conditions it is not equally affected throughout. We noticed differences in different parts of the mammary gland of the mouse during normal retrogression following pregnancy and lactation. Such inequalities might be in accord with the fact that the distribution of blood and lymph vessels, of ducts and of muscle tissue, is to some extent focal in character. Furthermore, it can be seen that in animals not adequately nourished the changes which take place in the fat tissue in which the gland is embedded are not everywhere the same. Moreover, hyaline tissue has a tendency to form especially around or near muscles. From here hyaline strands pass into the fat tissue and the distribution and direction of these strands seem to be determined at least partly by mechanical stress. It is, in part, upon such factors that the amount of hyaline tissue present around ducts and acini depends. The formation of small vacuolated fat tissue or of hyaline tissue around the ducts and acini may exert an injurious effect on these structures. To a certain extent therefore, the development of growth and secretory processes in the glands should not be expected to be uniform and to affect the whole gland equally, except under special conditions, and the focal development of gland tissue cannot under these circumstances be considered pathological. It is essentially this focal character of the growth processes in the mammary gland which determines the focal development of carcinoma in the mammary gland.

Summary

1. If injections of very large amounts of estrogen are given to mice during late stages of pregnancy, or at the beginning of lactation, and continued for various periods not exceeding 3 months, the retrogression of alveoli and ducts of the mammary gland which takes place normally following pregnancy and lactation is not prevented. However, localized processes of stimulation in the gland consisting of hypertrophy, mitotic growth, and secretion, which may affect either single acini scattered throughout the gland, or groups of acini of various sizes, are associated with these retrogressive changes, which latter predominate.

2. The changes observed in such mice under the influence of estrogen are, in principle, similar to those observed in cases of normal retrogression following lactation, except that the number and size of the active areas in the mammary gland are increased

after administration of estrogen. In both normal and experimentally stimulated mice the stimulation does not affect the whole gland in a uniform way, but is focal in character.

3. There is no significant difference in these effects of estrogen in strain A with a medium to high rate of cancer incidence and strain CBA with a low cancer rate.

4. It has not been found possible so far to produce a direct transition of the fully developed mammary gland of pregnancy or lactation into a carcinomatous gland by continued injection of very large doses of estrogen.

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The Incidence of Mammary Gland Carcinoma and Cancer Age in Mice Injected with Estrogen and in Noninjected Mice of Different Strains*

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In earlier investigations it has been established that the hereditary tendency to the development of carcinoma of the mammary gland differs greatly in different strains of mice (8, 9). It has been shown that a definite relation exists between the frequency with which mammary cancer appears in a certain strain and the age at which the cancer develops. In a general way, the cancer has a tendency to develop at an early age in strains with high incidence of tumors and at a later age in strains with low incidence of tumors. Certain peculiarities exist in certain strains. In some strains with low incidence of tumors, carcinoma of the mammary gland appears at an early period of life, and in some strains with high incidence of tumors it appears in older mice (4, 5, 6, 11).

More recently, Suntzeff, Burns, Moskop, and Loeb (10) have studied the incidence and age of appearance of mammary gland carcinoma in more closely inbred strains, some of which were derived from the strains of Miss Lathrop which had been used in our earlier investigations. In addition, the cancer rate and cancer age were compared in mice injected with various doses of estrogen and in noninjected (control) mice (10). In the meantime, we have continued these observations and we are now able to report on our findings in a much larger number of animals. However, in the investigations which were carried out in the course of the last 8 years the number of strains observed was much smaller than those used in our earlier studies. These strains [1] can be arranged in accordance with decreasing cancer incidence in the following order: (a) D and C3H, (b) A,[2] (c) New Buffalo, (d) Old Buffalo, (e) CBA, and (f) C57. We grouped the mice into the same three age classes as in our earlier investigations: age class I, 5-12 months; age class II, 13-17 months, and age class III, 18 months and over. Amounts of 100 to 200 rat units of estrogen,[3] dissolved in oil and injected once a week, were designated as large doses; 10 to 50 rat units of estrogen, dissolved in water and injected 5 or 6 times weekly, were designated as medium doses. Although the actual amount of estrogen injected per week may have been greater in this second type (medium doses) of experiments than in the first type (large doses), the estrogen in the medium doses was very much less effective because the estrogenic hormone in this case was dissolved in water instead of in oil and was therefore eliminated much more rapidly. The really effective dose was thereby much diminished. One to 10 rat units of this substance, dissolved in water and injected 5 or 6 times weekly, were designated as small doses.

I. Control Mice

Ages at death and cancer ages.—In Table I the percentages of mice which died with and without mammary gland tumors in the different strains and in the three age classes are compared, breeding and

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[1] Strains D, C3H, A, CBA, and C57 were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The Old and New Buffalo strains were obtained from Mr. Millard Marsh of the New York State Institute for the Study of Malignant Diseases.

[2] There is a discrepancy between the tumor rate in strain A as observed in our laboratory and in some other laboratories. The rate of tumor incidence in strain A has been lower in our laboratory and this lower rate has been consistent throughout a number of years. We have considered the cause of this difference on various occasions and no definite statement can be made. We do not think it probable that the nature of the diet is the responsible factor. Our mice receive Purina Dog Chow, a mixture of corn, oats, and sunflower seed, and once a week dry bread. They are kept in standard cages under the most hygienic conditions possible. In earlier experiments, Loeb and Lathrop, in some of their strains, had observed certain changes in the tumor rate of mice and there was reason for attributing these changes to genetic factors.

[3] We are indebted to Dr. Erwin Schwenk of the Schering Corporation for supplying us with crystalline dihydro-follicular hormone and Progynon-B (benzoic-acid ester of dihydro-follicular hormone).

nonbreeding mice being considered separately. The figures in parentheses are the percentages of nontumor or of tumor mice, with respect to the total number of breeding or nonbreeding female mice in each strain.

has not been possible to distribute the strain CBA mice according to the three age classes, but the ages of the breeding mice in which tumors developed were 9, 11 (4 mice), 12, 14, 16, 17, 21, 22, and 28 months. The average age of these mice was 15.2

TABLE I: AVERAGE AGE AT DEATH IN NONTUMOR MICE AND AVERAGE AGE OF TUMOR DEVELOPMENT IN TUMOR MICE

	Mice without tumors							Mice with tumors					
		Class I		Class II		Class III			Class I		Class II		Class III	
Strains	Total number of mice	Number died	Per cent of total	Number died	Per cent of total	Number died	Per cent of total	Total number of mice	Number died	Per cent of total	Number died	Per cent of total	Number died	Per cent of total
D														
Breeding	129 (34.4%)	89	69.0	32	24.8	8	6.2	246 (65.6%)	197	80.0	39	15.8	10	4.1
Nonbreeding	160 (75.8%)	86	53.9	41	25.6	33	20.5	51 (24.2%)	10	19.6	26	50.9	15	29.5
C3H														
Breeding	120 (40.1%)	98	81.66	22	18.34	0	0	179 (59.9%)	160	89.0	18	10.0	1	0.6
Nonbreeding	115 (80.9%)	56	48.7	54	46.9	5	4.4	27 (19.1%)	9	33.3	12	44.4	6	22.3
A														
Breeding	211 (57.0%)	114	54.0	60	28.4	37	17.5	157 (43%)	112	71.3	38	24.2	7	4.5
Nonbreeding	199 (97%)	29	14.5	84	42.2	86	43.3	6 (3%)	..	...	3	50.0	3	50.0
New Buffalo														
Breeding	88 (71.5%)	24	27.3	30	34.0	34	38.7	35 (28.5%)	9	25.7	14	40.0	12	34.3
Nonbreeding	87 (83.6%)	18	20.7	23	26.4	46	52.9	17 (16.4%)	2	11.8	4	23.5	11	64.7
Old Buffalo														
Breeding	81 (93.1%)	34	42.0	17	20.9	30	37.1	6 (6.9%)	..	...	4	66.6	2	33.3
Nonbreeding	235 (96.7%)	87	37.0	50	21.2	98	41.8	8 (3.3%)	1	12.5	3	37.5	4	50.0
CBA														
Breeding	167 (93.3%)	88	52.7	55	32.9	24	14.4	12 (6.7%)	6	50.0	3	25.0	3	25.0
Nonbreeding	140 (99.3%)	68	48.6	46	33.0	26	18.6	1 (0.7%)	..	...	..	...	1	100.0
C57														
Breeding	305 (100%)	163	53.4	73	23.9	69	22.6	...	..	...	..	...	..	...
Nonbreeding	112 (100%)	45	40.2	29	25.9	38	33.9	...	..	...	..	...	..	...

In regard to strain CBA, the figure for breeding, nontumor mice could not be ascertained with complete accuracy, but it is probably 167. There were among them 12 mice with mammary gland tumors. The tumor incidence would therefore be 6.7 per cent. Among 140 female nonbreeding mice there was one mouse with a tumor, an incidence of 0.7 per cent. It

months. One tumor appeared in a nonbreeding mouse at the age of 24 months. In strain C57, no tumor was observed.

The percentages of nontumor and of tumor mice in the three age classes shown in Table I have, therefore, been determined without regard to the number of mice which were alive in each age class.

Incidence of tumors in breeding and nonbreeding mice.—In Table II a summary is given of the total and percentage figures of the breeding and nonbreeding mice in each strain. The percentages of tumors in nonbreeding mice in the various strains as compared with the percentages of tumors in breeding mice, if the percentages of tumors in breeding mice are set at 100 per cent, were—D: 36.9 per cent; C3H: 31.8 per cent; A: 7 per cent; New Buffalo: 57.5 per cent; Old Buffalo: 47.8 per cent; CBA: 11.8 per cent. In the Buffalo strains the percentage was highest; here tumors occurred in nonbreeding mice about one-half as frequently as in breeding mice. The figure was lowest in the A strain, where only 7 tumors occurred in nonbreeding mice for each 100 tumors in breeding mice. Also, in CBA, the proportion was much less (11.8 per cent) than in the Buffalo strains. In strains D and C3H, the proportion was about 1 tumor in nonbreeding to 3 tumors in breeding mice. There was, therefore, a great difference in different strains as to the relative frequency of tumors in breeding and nonbreeding mice, a finding that agrees with our earlier findings (3, 7).

Table II: Total Number and Percentages of Tumors in Breeding and Nonbreeding Mice of Each Strain

Strains	Number of breeding mice	Percentage of tumors	Number of nonbreeding mice	Percentage of tumors	Percentage of tumors in nonbreeding mice to 100 tumors in breeding mice
D	375	65.6	211	24.2	36.9
C3H	299	59.9	142	19.1	31.8
A	368	43.0	205	3.0	7.0
New Buffalo	123	28.5	104	16.4	57.5
Old Buffalo	87	6.9	243	3.3	47.8
CBA	202	5.9	141	0.7	11.8
C57	305	0	112	0	...

Discussion.—In Table I the order of the percentages of the total number of breeding mice in which tumors appeared in the different strains, without respect to the age of the mice, declined as follows: D, C3H, A, New Buffalo, Old Buffalo, CBA, and C57. In nonbreeding mice the number of mice with tumors was less than in breeding mice, but the order was the same, except that in strain A the percentage was lower than in the New Buffalo strain and about of the same order as in the Old Buffalo strain. If we compare the percentages of mice with tumors in the different age classes without respect to the total number of mice available for the development of tumors in each age class, we find that among breeding mice the tumors appeared earlier in the C3H than in the D strain; these two strains were followed in declining order by strains A, New Buffalo, and Old Buffalo. In the CBA strain the tumors appeared earlier than in the New Buffalo strain and later than in the A strain. However, in making this comparison we have to consider that in the Old Buffalo and CBA strains the total number of tumors in breeding mice was relatively small.

In nonbreeding mice the highest age incidence of tumors is shifted in the direction of the later ages. In the high tumor strains (D, C3H, A) the highest incidence was in the second instead of in the first age class, as in breeding mice. In the A strain the age incidence in age classes II and III was the same, while in the strains with the lower rate of tumor incidence (New Buffalo, Old Buffalo, CBA) the highest incidence was in the third age class. In Table II the percentages of mammary gland tumors in breeding and in nonbreeding mice are compared.

In general, the greater the total tumor incidence in a certain strain, the greater was the tendency for the tumors to appear early in life. The intrinsic factor that spurs on the tumor development also tends to make the tumors appear earlier in life. When this factor is stronger, the cancerous transformation is accomplished in a larger number of individuals and at an earlier date, that is, after a shorter action. This agrees with our earlier findings, except that we noted then the additional fact that in some strains or families special conditions were active which made the tumors appear relatively early in life, although the tumor incidence was not high, and, conversely, in some strains with a high incidence the tumors appeared relatively late in life. However, in these earlier investigations our conclusions were based on the comparison of a much larger number of strains and families than those at our disposal in these more recent investigations.

In the nonbreeding mice, in which stimuli to tumor development are weaker than in breeding mice, the tumors appeared accordingly at a later period of life, and this late incidence was most marked in the Buffalo and CBA strains, in which the total tumor incidence among the nonbreeding mice was lowest. In the A strain, where in nonbreeding mice the inci-

dence was very low, the age distribution was similar to that of the Buffalo mice.

As to the percentages of mice in the various strains which died without tumors, two factors must be considered.[4] In the first place, these figures are influenced by the tumor incidence in the various age classes; the greater the number of mice which die in the first age class with tumors, the smaller is the number of mice left in the age classes II and III. This factor will be of more significance among the breeding than among the nonbreeding mice, because more breeding mice die from mammary gland carcinoma in the first and second age classes than nonbreeding mice. This factor will differ also in accordance with the difference in the tumor incidence among breeding and nonbreeding mice in the different strains. The fact that among the breeding mice of strains D, C3H, and A the percentage of mice dying without tumors is greatest in the first age class and least in the third age class, is partly due to the great diminution in the number of mice in the later age classes which remained alive. However, in strain C57, which is free of tumors of the mammary gland, the percentage of mice dying in the first age class is greater than the percentage of mice dying in the later age classes, although the preponderance in the first age class is not so great as in strains D and C3H. This indicates that other factors also may be responsible for the high death rate in the first age class.

In addition, in all strains, the breeding mice tended to die at an earlier age than the nonbreeding mice. This was also true in strain C57, in which the tumor rate, being zero, does not influence the age of death. This agrees with our earlier observation that the nonbreeding mice tend to live longer than the breeding mice. An exception to this rule is found in the Old Buffalo strain, where the proportion of mice in the different age classes dying without tumors is about the same in breeding and in nonbreeding mice.

TABLE III: TUMOR PERCENTAGES IN 3 AGE GROUPS OF 6 STRAINS OF CONTROL MICE *

Number of mice		Class I			Class II			Class III		
		Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
586	Strain D									
	Breeding	375	197	52.7	88	39	44.3	18	10	55.5
	Nonbreeding	211	10	4.7	115	26	22.6	48	15	31.2
441	Strain C3H									
	Breeding	299	160	53.5	41	18	44.0	1	1	100.0
	Nonbreeding	142	9	6.3	77	12	15.6	11	6	54.5
573	Strain A									
	Breeding	368	112	30.4	142	38	26.6	44	7	16.0
	Nonbreeding	205	0	0	176	3	1.7	89	3	3.37
227	Strain New Buffalo									
	Breeding	123	9	7.3	90	14	15.5	46	12	26.0
	Nonbreeding	104	2	1.9	84	4	4.76	57	11	19.3
330	Strain Old Buffalo									
	Breeding	87	0	0	53	4	7.5	32	2	6.2
	Nonbreeding	243	1	0.4	155	3	1.93	102	4	4.0
343	Strain CBA									
	†Breeding	202	6	3.0	88	3	3.4	30	3	10.0
	Nonbreeding	141	0	0	73	0	0	27	1	3.7
417	Strain C57									
	Breeding	163	0	0	73	0	0	69	0	0
	Nonbreeding	45	0	0	29	0	0	38	0	0

* Total number of mice—2,917.
† The age of only 167 of the breeding mice was known.

Tumor percentages in different age groups and strains.—The data given in Table III confirm the conclusions at which we arrived on the basis of data in Tables I and II.

Table III shows the percentages of the total number of mice available at the beginning of each age class among breeding and nonbreeding mice which die with tumors. The breeding mice of the strains with the highest tumor incidence (D and C3H) showed no great difference between the percentage of tumors in the various age classes.

Discussion.—The factors leading to the development

[4] The percentages of mice dying with or without tumors in the three age classes were calculated on the basis of the total number of mice in each strain.

of tumors are here so potent that the percentage in the first age class is about as high as in the later age classes. Apparently the mammary gland in these strains is so near the threshold to cancer formation that in many individuals the stimuli furnished during a relatively short period of sexual maturity are sufficient to induce this transformation of normal into cancer tissue; but in many other individuals a longer-lasting action of the growth stimuli is necessary to accomplish this effect and a certain number of D mice in age class III died without tumors. In strain A there is an indication that the tumor incidence among breeding mice declines somewhat with advancing age. It seems that in certain individuals the resistance of these strains to the development of mammary gland carcinoma.

The nonbreeding mice differ from the breeding mice in that in the former the tumor incidence increases steadily with increasing age. In the nonbreeding mice the conditions are less favorable for the development of mammary gland tumors than among the breeding mice. This is presumably due to the fact that in the nonbreeding mice the amount of stimulation reaching the mammary gland is less than in the breeding mice. In order to reach the threshold of stimulation required for the cancerous transformation, it is necessary for the stimuli to act over a longer period of time in the nonbreeding mice.

TABLE IV: PERCENTAGE OF MICE WITH TUMORS IN TREATED AND CONTROL GROUPS

Strains		Percentage of mice with tumors among all mice injected with estrogen for 4 months and upwards: Total for all mice	Large doses	Medium doses	Small doses	Total incidence in controls	
D	I *	55.8	50	61.1	75	Breeding	65.6
	II †	58.5	54.2	61.5	75	Nonbreeding	24.2
						Total	50.6
C3H	I *	57.3	58.3	55.5	..	Breeding	59.9
	II †	63.6	69.2	55.5	..	Nonbreeding	19.1
						Total	46.7
A	I *	31	20.4	47.1	25	Breeding	43
	II †	41.5	32.2	53.3	25	Nonbreeding	3
						Total	28.4
New Buffalo	II †	20.8	22.2	16.6	..	Breeding	28.5
						Nonbreeding	16.4
						Total	22.9
Old Buffalo	II †	19	30.7	...	..	Breeding	6.9
						Nonbreeding	3.3
						Total	4.2

* I—All mice 5 months old or older and injected for 4 months or longer are included.
† II—Only mice injected for 5 months or longer are included. In New Buffalo and Old Buffalo strains all the injected mice received estrogen for 5 months or longer.

there are conditions which oppose the development of mammary cancer and which even a long-continued action of cancer producing growth stimuli may not be able to overcome.

In the New and Old Buffalo strains, in which the hereditary tendency to the development of mammary gland carcinoma is less potent, the tumor incidence increases with an increase in length of period of stimulation. The same holds good in the case of the CBA strain, in which the total tumor rate was still lower. We may conclude that the sum of the stimuli acting on the mammary gland must be greater in strains with a low hereditary tendency than in strains with high incidence of tumor in order to overcome

II. MICE INJECTED WITH VARIOUS DOSES OF ESTROGEN

Effect of estrogen on incidence of tumors.—Table IV shows that in strains D, C3H, A, and New Buffalo, nonbreeding mice injected with estrogen reached approximately the tumor incidence of breeding, non-injected mice. In the Old Buffalo strain, the injected mice attained a higher incidence, but in this strain (as well as in the New Buffalo strain) the number of mice in which tumors developed was small. In strains CBA and C57, tumors were not observed in the injected mice. In strains D, C3H, and A there was no significant difference between the incidence of tumors in mice injected with large doses of estrogen

and those injected with medium or small doses. In strain A, the incidence of tumors was highest in the animals injected with medium doses, but in the Buffalo strains the incidence appeared to be higher in those injected with large doses than in those injected with small doses of estrogen.

Discussion.—It should not be concluded from these figures that, through injections of estrogen, it is not possible to raise the incidence of mammary gland carcinoma above that observed in breeding control mice. There are indications that the injection of 100 rat units of estrogen twice weekly is more effective than that of 100 rat units once weekly; but our figures in these experiments are too small to make possible a definite conclusion in this respect.

While thus, if we merely consider the incidence of mammary gland carcinoma, the amount of estrogen injected into mice with high tumor rates (strains D, C3H, A) did not exert a significant effect on the incidence of mammary gland carcinoma, and there was no proportionality between the doses of estrogen used and the frequency with which tumors appeared, it was possible that the quantities of estrogen administered affected the age at which the carcinomas developed. Therefore, we classified the mice in the various strains according to the doses of estrogen given and the percentage of tumors that appeared in each of the three age classes.

Relation of age classes to effects of estrogen.—It is seen that when large doses of estrogen were given in the high tumor rate strains, the largest number of tumors appeared in the first age class (Table V). In the second and third age classes relatively few mice were left, and the determination of the tumor rates became therefore less trustworthy in these age classes. In the D and A strains the percentage of tumors was lower in the later age classes than in age class I, while in strain C3H, in which the largest number of tumors appeared likewise in age class I, the percentages of tumors happened to be higher in age classes II and III.

TABLE V: PERCENTAGE OF TUMORS IN ALL AGE CLASSES INJECTED WITH ESTROGEN *

Doses	Class I			Class II			Class III		
	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
STRAIN D									
Large	26	12	46.0	5	1	20.0	2	0	0
Medium	13	1	7.7	8	3	37.5	4	4	100
Small	..	..	...	4	3	75.0	..	..	....
STRAIN C3H									
Large	48	24	50.0	4	3	75.0	1	1	100
Medium	27	7	25.9	16	8	50.0	..	..	....
STRAIN C3H + D									
Large	74	36	48.6	9	4	44.4	3	1	33.3
Medium	40	8	20.0	24	11	45.9	4	4	100
Small	..	..	...	4	3	75.0	..	..	....
STRAIN A									
Large	49	10	20.4	2	0	0	..	..	....
Medium	34	4	11.8	20	6	30.0	11	6	54.6
Small	..	..	...	4	1	25.0	..	..	....
STRAIN NEW BUFFALO									
Large	18	1	5.5	3	2	66.6	1	1	100
Medium	6	0	0	5	1	20.0	..	..	....
STRAIN OLD BUFFALO									
Large	13	2	15.4	4	2	50.0	..	..	....
Medium	..	..	...	2	0	0	..	..	....
Small	..	..	...	8	0	0	..	..	....
STRAIN NEW BUFFALO + OLD BUFFALO									
Large	31	3	9.7	7	4	57.1	1	1	100
Medium	8	0	0	7	1	14.3	..	..	....
Small	..	..	...	8	0	0	..	..	....

* The number of mice in class I includes also mice in classes II and III. All mice, irrespective of duration of injections, are included.

If we combine the figures for strains D and C3H, the highest incidence is found in age class I; it is slightly less in age classes II and III. While the figures for the Buffalo strains are relatively small, still they indicate that here the tumor incidence is lower in the first age class than in age classes II and III. With a low degree of hereditary tendency to cancer, the growth stimuli must act over longer periods of time in order to be effective.

Discussion.—If, instead of large doses, medium and small doses of estrogen are given, a shift in the maximum tumor incidence towards the oldest age classes takes place in all of the strains. This means that in order to obtain the same effect with smaller, as with large doses, the small doses have to act over longer periods of time. While the incidence of tumors is not proportional to the doses of estrogen injected—except perhaps in the Buffalo strains, where the incidence seems to be greater with large than with medium doses—the tumors appear earlier in life in mice injected with large doses than in those injected with medium or small doses. In Table V, the injected mice in each strain are classified according to the doses of estrogen given and the percentage of tumors which appear in each age class. In strains D and A, the tumors appear earlier the larger the dose injected. In strain C3H this is also the case, although it is not quite so evident as in strains D and A. In strain C3H, the normal mice are perhaps so near the threshold of tumor development that medium doses are here relatively more effective than in strains D and A. In the Buffalo strains, which are further removed from this threshold, the incidence of tumors is greatest in the second age class, and this applies to mice injected with large as well as to those injected with medium doses. In these strains the stimulus must act over a longer time before the resistance to tumor growth is overcome.

Both Tables IV and V show that the incidence of mammary gland carcinoma in the mice treated with estrogen is less in strain A than in strains D and C3H. This accords with the lower tumor incidence in the control breeding mice in strain A as compared with strains D and C3H. The tumor incidence in the Buffalo strains is lower than in A. Then follow, in decreasing order, strains CBA and C57. Lacassagne (2) and others have observed that it was possible to produce mammary gland carcinoma in male mice belonging to strains with high incidence of tumors, but not in those belonging to strains with very low tumor rates. We now find that there exists a general correspondence between the incidence of mammary gland carcinoma in breeding mice of the various strains and the frequency and rapidity with which this type of cancer appears in female as well as in male mice injected with estrogen. Such a correspondence is in accordance with expectations, because we know that both types of mammary carcinoma—the so-called spontaneous carcinoma and the one experimentally induced—owe their origin to the same cause; namely, to an interaction of hereditary factors, which largely determine the degree of responsiveness of the mammary gland tissue to stimuli of an endocrine nature, and of ovarian or related hormones. The same order of incidence applies also to the nonbreeding mice, except that in this case the incidence of spontaneous tumors in strain A is too low. We may interpret this peculiarity of the A strain as due to the character of the curve which represents the intensity of reaction of the mammary gland to various quantities of stimuli. While these curves seem to take an approximately parallel course in the various other strains which we have examined, the curve of the A strain apparently differs from the others, insofar as at a certain point, which lies between the degree of stimulation exerted by the ordinary amount of ovarian hormone active in nonbreeding mice and the amount effective during pregnancy, and perhaps also during lactation, a steep rise occurs in the curve representing the response of the tissue to the amount of stimulation. In our earlier investigations we had observed peculiarities in various strains concerning the relative proportions of individual mice of a certain strain, which became cancerous under the influence of endogenous hormone action, and the age at which the cancerous condition developed. As stated above, in the much smaller number of strains which we have studied here these differences did not appear, but certain other individual characteristics were found in these strains.

Duration of injections and percentages of tumors.—In Tables VI to XI, the mice belonging to those strains in which injections of estrogen increased the cancer rate and accelerated the appearance of tumors are divided into four groups, according to the duration of the injections which induced the tumor formation.

Discussion.—Concomitantly with the differences in duration there occurred also differences in the ages of the mice in the various groups. There enter in these tables, therefore, not only the length of the time during which the injections were given, but also differences in the ages of the injected mice as a complicating factor. Those mice which had received injections during the longest periods (group IV) were also the oldest mice; they belonged to the age classes II and III, while those which had received injections for only 3 to 12 months belonged either to the first or to the later age classes, according to the time of life at which the tumor appeared. There is another complicating factor which has to be considered

in the evaluation of these tables. In mice injected for a certain length of time, tumors do not necessarily appear at the end of the period of injections, but a latent period may occur between the end of the injections and the time when the tumor becomes be longer the shorter the period of injections. Making allowance for these various complications, we may interpret Tables VI and VIII.

In strains D (Table VI) and A (Table VIII) the occurrence of tumors in mice injected for 3 to 4

TABLE VI: STRAIN D: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS (OVARIECTOMIZED MICE INCLUDED)

(All injected mice, irrespective of age, are included)

Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
FEMALES											
100-200 r.u. of estrogen											
*16	0	0	11	5	45.5	4	2	50	2	0	0
10-50 r.u. of estrogen											
..	..	...	13	1	7.6	8	2	25	6	5	83.3
1-5 r.u. of estrogen											
..	..	...	..	..	...	4	1	25	2	2	100
MALES											
100-200 r.u. of estrogen											
21	0	0	13	3	23.1	5	2	40	3	1	33.3

* The number of mice in group I includes also the mice in groups II, III, and IV; the number of mice in group II includes also mice in groups III and IV; the number of mice in group III includes also mice in group IV.

TABLE VII: STRAIN C3H: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS (OVARIECTOMIZED MICE INCLUDED)

(All injected mice, irrespective of age, are included)

Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
FEMALES											
100-200 r.u. of estrogen											
*39	1	2.6	29	14	48.3	7	3	42.9	2	2	100
10-50 r.u. of estrogen											
..	..	...	10	2	20	7	3	42.9	4	4	100
MALES											
100-200 r.u. of estrogen											
11	2	18.2	9	4	44.5	2	1	50	1	1	100
10-50 r.u. of estrogen											
..	..	...	17	0	0	16	2	12.5	12	4	33.3

* The number of mice in group I includes also the mice in groups II, III, and IV; the number of mice in group II includes also mice in groups III and IV; the number of mice in group III includes also mice in group IV.

visible to the naked eye. This has been shown for mice injected with estrogen by Burns and Schenken (1), and we had previously noted the existence of such a latent period in otherwise normal, but ovariectomized, noninjected mice. This latent period will months is zero or very low; it increases greatly in mice injected for 5 to 8 months, and reaches a maximum in mice injected for 9 to 12 months, after which it declines sharply in mice injected for longer than 12 months. The later groups (groups II, III, and IV)

include not only the mice injected for a certain length of time, but also the mice which became cancerous at a time when the injections had ceased as a result of the earlier injections of estrogen, or as a result of the action of endogenous estrogen. Strain C3H

In those mice in which the hereditary factors which promote the development of mammary gland cancer are less potent, the incidence rises more steeply in the higher groups III and IV. Likewise, if the stimulus is less strong, as it is in mice injected with medium

TABLE VIII: STRAIN A: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS (OVARIECTOMIZED MICE INCLUDED)

(All injected mice, irrespective of age, are included)

Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
FEMALES											
100-200 r.u. of estrogen											
*40	0	0	24	3	12.5	5	3	60	..	..	...
10-50 r.u. of estrogen											
30	0	0	29	4	13.8	23	4	17.4	16	8	50
1-5 r.u. of estrogen											
..	..	...	..	..	...	..	..	...	4	1	25
MALES											
100-200 r.u. of estrogen											
9	0	0	7	1	14.3	3	3	100	..	..	...

* The number of mice in group I includes also the mice in groups II, III, and IV; the number of mice in group II includes also mice in groups III and IV; the number of mice in group III includes also mice in group IV.

TABLE IX: STRAIN NEW BUFFALO: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS

(All injected mice, irrespective of age, are included)

Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
FEMALES											
100-200 r.u. of estrogen											
*10	0	0	9	1	11.1	1	0	0	3	3	100
10-50 r.u. of estrogen											
..	..	...	..	..	...	..	..	...	6	1	16.6
MALES											
100-200 r.u. of estrogen											
..	..	...	..	..	...	9	0	0	..	..	...

* The number of mice in group I includes also the mice in groups II, III, and IV; the number of mice in group II includes also mice in groups III and IV; the number of mice in group III includes also mice in group IV.

differs from strains D and A, inasmuch as the tumor incidence was highest in mice of group IV, which had been injected for longer than 12 months. In strain C3H, also, the curve rises steeply in group II, and reaches its peak in group III. But in the same strain, tumors also occurred in group I, in contrast to the absence of tumors in strains D and A.

or small doses, a longer application of the stimulus is necessary, the maximum occurring in group IV, and here the rise is steepest between group III and group IV. These relations are seen in Table XI, where the tumor incidence in the four groups of the combined strains with high tumor incidence (D, C3H, A) and of the two Buffalo strains is shown. Also, the

incidence in mice injected with large and smaller doses of estrogen is here compared.

Comparison of tumor incidence according to sex.—As to the difference between female and male mice, Table XII, in which mice belonging to strains D, C3H, and A and injected with large doses of estrogen are combined, shows that there is no essential difference between the tumor incidence in the two sexes.

TABLE X: STRAIN OLD BUFFALO: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS (OVARIECTOMIZED MICE INCLUDED)

(All injected mice, irrespective of age, are included)

Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
FEMALES											
100-200 r.u. of estrogen											
*11	0	0	4	0	0	3	3	100	..	..	...
10-50 r.u. of estrogen											
..	..	...	..	..	...	..	..	...	2	0	0
0.33-0.5 r.u. of estrogen											
..	..	...	..	..	...	8	0	0	..	..	...
MALES											
100-200 r.u. of estrogen											
5	0	0	..	..	...	..	..	...	3	1	33.3

* The number of mice in group I includes also the mice in groups II, III, and IV; the number of mice in group II includes also mice in groups III and IV; the number of mice in group III includes also mice in group IV.

TABLE XI: PERCENTAGE OF TUMORS IN MICE WHICH WERE DIVIDED INTO 4 GROUPS ACCORDING TO DURATION OF INJECTIONS

Doses	Group I 3-4 mos. injections			Group II 5-8 mos. injections			Group III 9-12 mos. injections			Group IV Over 12 mos. injections		
	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent	Number of mice	Number of tumors	Per cent
COMBINED STRAINS D, C3H, AND A												
Large	136	3	2.2	93	30	32.2	26	14	54	8	4	50
Medium and small	78	0	0	77	7	9.1	62	12	19.3	44	24	54.5
COMBINED STRAINS NEW AND OLD BUFFALO												
Large	35	0	0	25	1	4	19	3	15.7	6	4	66.6
Medium and small	..	..	...	..	..	...	16	0	0	8	1	12.5

TABLE XII: COMPARISON OF TUMOR INCIDENCE IN MALES AND FEMALES

	Percentage of tumors in female mice	Percentage of tumors in male mice
Group I	1	5
Group II	34.4	27.6
Group III	50	60
Group IV	50	50

Discussion.—It might have been expected that the incidence would be greater in female than in male mice, because in the former the endogenous ovarian hormones are active in addition to those of exogenous origin. However, the injection of large doses of estrogen tends to reduce the production of endogenous estrogen, and this may perhaps explain why the tumor incidence is about as high in male as in female mice. It would be of interest to compare the tumor incidence in male and female mice which have been injected with medium and small doses of estrogen, and also in mice belonging to strains with less tendency to the development of tumors. Our figures are not large enough for the purpose of such a comparison, but they indicate that perhaps the incidence in the latter cases is higher in female than in male mice.

SUMMARY AND CONCLUSIONS

1. There exists, in general, a parallelism between the cancer incidence of the mammary gland in breeding control mice of various strains and the readiness

with which the cancer rate can be increased in these strains in nonbreeding female mice, or the occurrence of mammary gland carcinoma can be induced in male mice by injections of estrogen. This parallelism is due to the fact that in all of these cases we have to deal with the same mechanism; namely, the production of carcinoma through the action of endogenous or exogenous ovarian hormones acting on a substratum with a definite inherited degree of growth tendency in the mammary gland.

2. The proportion between the cancer incidence in breeding and nonbreeding control mice varies greatly in different strains, and these differences must be considered as due to an inherited strain characteristic, a conclusion which confirms our earlier observations made with different strains of mice.

3. In breeding control mice belonging to strains with the highest incidence of mammary gland carcinoma, the cancer rate was approximately as high in the first as in the later age classes. In strains with a weaker inherited tendency to the development of mammary gland carcinoma, and in nonbreeding mice even of high tumor rate strains, the cancer incidence increases in the direction from the first to the third age class. This indicates that cancer in general appears the earlier in life the greater the intensity with which the causative factor acts.

4. A similar relationship is observed in mice treated with exogenous estrogen—the stronger the dose of estrogen injected and the greater the inherited tendency to cancer, the earlier the mammary gland carcinoma appeared in the various strains.

5. No significant difference in the incidence of mammary gland carcinoma was observed in male and nonbreeding female mice injected with large amounts of estrogen and belonging to strains with a strong hereditary tendency to the development of this type of cancer.

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Tumor Inhibition Associated with Secretory Changes Produced by Estrogen in a Transplanted Mammary Adenocarcinoma of the Rat*

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The relationship of the estrogenic hormone to malignant tumors, especially carcinoma of the breast in certain strains of mice, has been extensively investigated in recent years. Nevertheless it is still considered a debatable question whether the estrogens can be compared with the direct-acting carcinogenic hydrocarbons in their relationship to carcinogenesis. Cancer developing in a tissue stimulated abnormally by an excessive amount of a physiologic agent may be a complication arising from the fortuitous action of another agent or factor.

The effect of the sex hormones on existing malignant and benign tumors of the reproductive system or of extragenital origin has been the subject of a number of studies. As is commonly observed, differences in animal stocks, tumors, the hormones employed, and the technic of administration have produced apparent discrepancies in the results obtained. The Zondeks and Hartoch (20) asserted that pituitary gonadotropic hormone inhibited the growth of the Ehrlich adenocarcinoma of the breast in mice. Krehbiel, Haagensen, and Plantenga (13) could not confirm this effect on mouse sarcoma 180, and Bischoff and Maxwell (3) also found this hormone to be without effect on both sarcoma 180 and a transplantable rat tumor (R 10). Gilmour (9) applied a 0.01 per cent solution of ketohydroxyestrone in chloroform to the skin of mice inoculated with breast cancer or sarcoma 180, and concluded that estrogen had some inhibitory action on tumor growth. She ascribed this effect to a general loss of weight. The retardation in tumor growth in her small groups of animals, however, did not occur consistently, nor was there any relationship between the degree of tumor inhibition and the dosage of hormone administered. Bischoff and Maxwell (3) found no effect of the estrogenic hormone (prepared from ether washings of the urine of pregnancy) on sarcoma 180 when administered in doses of 130 rat units daily for 15 days. Nathanson, Salter, and Wilson (16, 17) reported recently on the effect of estrone in a large series of mice of 2 inbred strains bearing sarcoma 180. The hormone directly inhibited tumor growth in one strain, C57 black, as shown by the smaller size of tumors in treated animals, while no effect occurred in a second strain, Bagg albino, strain A. Moreover, the resistance against sarcoma 180 produced by preliminary inoculation of the tumor in the tail was enhanced by the administration of 50 to 200 I. U. of estrone daily during the development of tumors in the tail and subsequent test tumors in the groin. This effect occurred in both strains of mice. The operation of complicating factors, however, obscures the possible relationship between the degree of immunity and the effects of hormones. Tumor immunity, in addition to appearing only in strains of animals genetically unrelated to the original tumor-bearer and, therefore, in which tumor growth is subject to irregularity, is extremely variable and frequently undependable in its reproducibility. According to these authors castration appeared to decrease tumor resistance in females. Testosterone had no effect.

Benign mammary tumors appear distinctly amenable to the influence of hormones. Heiman and Krehbiel (12) reported that estrogen, especially in combination with the anterior pituitary gonadotropic hormone, favored the growth of transplanted fibroadenoma in the rat. Heiman (10) later reported a similar effect in castrates. Androgen on the other hand exerted an inhibitory effect (11). Subsequently Mohs (14) reported suppression of the glandular elements and an increase in the fibrous component of transplantable mammary fibroadenoma in the rat as the result of the action of the androgenic hormone. Wright, Klinck, and Wolfe (18) confirmed the effect of the female sex hormone in rats with spontaneous fibroadenoma. Emge, Murphy, and Schilling (7, 15), however, found that theelin, administered for short or long periods, exerted no influence on benign transplantable mammary tumors in the rat.

The purpose of the present communication is to describe alterations produced by estrogen in the growth and structure of a transplantable adenocarcinoma of the rat's breast, and to record the absence of effect of pregnancy and lactation, castration, and the androgenic hormone on this tumor. The tumor employed, R 2426, is a well differentiated adenocarcinoma which has been described previously (6). Papillary and small cystic zones are frequently evident in the tumor. It arose spontaneously in a female rat of the August strain, is transplantable in 100 per cent of the animals of the same strain, but rarely grows in unrelated stocks. The high degree of specificity, regularity, and predictability of its growth and minimal amount of necrosis make this tumor particularly suitable for experimental observations. No difference was observed in its rate of proliferation or morphologic characteristics in animals of either sex ranging from the extremely young to the extremely old. It may be concluded, therefore, that it is not susceptible to the normal variations in hormone content of the body.

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Experimental

Inhibitory effect of the estrogenic hormone on tumor growth.—Rats of both sexes, varying in age from 4 to 7 months, were given estradiol benzoate,[1] 1,000 rat units (0.166 mgm.) in 0.1 cc. sesame oil alternately in each axilla twice weekly. Two injections were administered during the week prior to implantation of a 0.003 gm. tumor fragment on the right side of the abdomen at a considerable distance from the injected hormone. There were two exceptions to this general procedure:

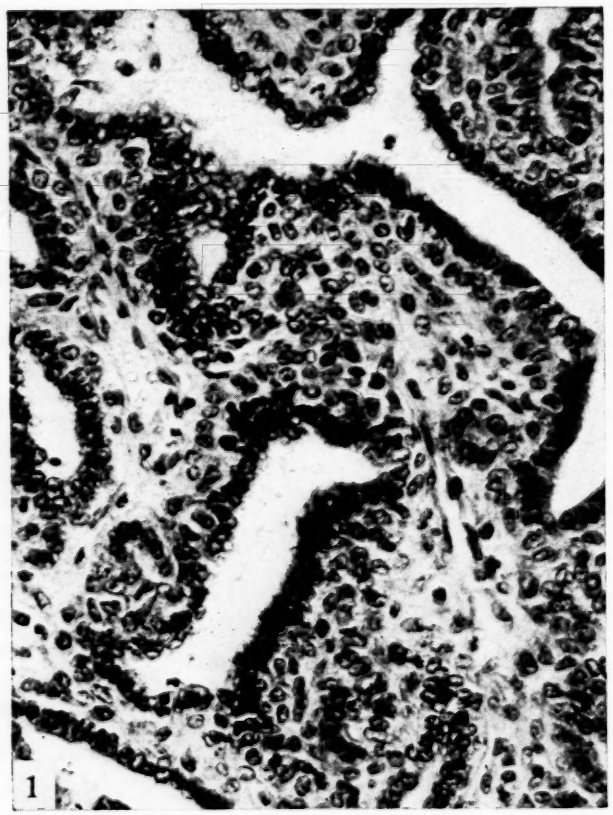


Fig. 1.—Typical appearance of mammary adenocarcinoma (R 2426) of the rat in the 14th transplant generation. Mag. × 375.

11 animals received estradiol dipropionate,[1] 0.2 mgm. twice weekly in 0.1 cc. sesame oil, and in 8 animals the injections of the benzoate were begun on the day of tumor transplantation. Since the effects of the hormone were comparable in each instance all animals were considered as constituting a single group of observations.

The tumors were measured in their two largest diameters and charted at weekly intervals, the first charting being made after 14 days. Identical and barely palpable growths were present at that time in both control and treated animals. Subsequent observations revealed a definite inhibitory influence of the hormone on tumor proliferation, but in no instance was there total suppression of neoplastic growth.

In Table I the mean tumor diameters of treated and control animals are compared. The mean of the two largest diameters of tumors has been accepted as a valid measure of computing their size, especially since transplanted subcutaneous tumors generally show the least amount of proliferation in their depth. The tumors were from 4 successive transplantations and the measurements given are those computed 44 to 49 days after implantation in animals that received 12 to 14 injections of estrogenic hormone. This limit was established arbitrarily as some control tumors began to show ulcerative changes at this time. Injections were continued in a number of treated animals and their tumors were consistently smaller than those in surviving control rats. The mean tumor size in estrogenized rats is approximately one-half that in untreated animals and the difference is statistically significant. Female and male rats are grouped together, as the influence of the excessive amount of estrogen was comparable in both sexes. The age of the animals had no determining influence on the effect of the estrogen on tumors.

Table I: Mean Diameters of Transplantable Carcinoma of the Rat Breast (R 2426) in Control and Treated Rats, 44 to 49 Days after Transplantation, 12 to 14 Injections of Estrogen in Treated Animals

	No. rats	Tumor diameter in mm., mean ± P.E.	Difference ± P.E.	Difference / P.E. difference
Control . . .	37 (21♀, 16♂)	25.00 ± 0.65	. .	. .
Treated . .	39 (22♀, 17♂)	13.00 ± 0.51	12.00 ± 0.82	14

The known general effects of an excessive supply of estrogen were readily apparent in both sexes. In females mammary secretion could usually be expressed from the nipples following 6 to 7 injections. Uterine hyperplasia was prominent, and in males a decrease in size of the genitalia attested the effect of the hormone. Beginning enlargement of the hypophysis occurred in both sexes. Although the rats withstood prolonged estrinization satisfactorily, a deleterious effect undoubtedly resulted. Their fur appeared unhealthy and despite access to liberal amounts of food (mixture of one-half Purina dog chow and one-half Rockland rat chow, fresh carrots weekly, and water as desired) loss of weight occurred. This is illustrated in Table II and is particularly evident on comparing

[1] Estradiol benzoate was supplied by Dr. Louis Klein of Hoffman-La Roche, Inc., estradiol dipropionate by Dr. R. MacBrayer of Ciba Pharmaceutical Products, Inc., and testosterone propionate by Dr. Erwin Schwenk of the Schering Corporation. The author expresses his thanks for their generous cooperation.

the body weights of the groups of estrinized and control animals fed an identical diet. The final body weights are those recorded 52 to 54 days after the first injection of estrogen. This interval corresponds with the time of recording the tumor diameters in Table I, as rats with tumors received estrogen injections in the week prior to transplantation.

Zondek (19) reported that the administration of 5,000 mouse units of estrogen twice weekly to juvenile rats produced impairment of body growth after 3 to 4 weeks. Freudenberger and Clausen (8) demonstrated that a significant decrease in the body weight of female rats injected daily with 200 I. U. (60 rat units) of theelin occurred after 6 to 10 days, when treatment was begun in animals 21 days of age. In the experiments of Nathanson and Salter (16), however, no decrease was observed in the body weight of C57 black male mice receiving 50 to 200 I. U. estrone daily for 3 weeks. These authors conclude that any inhibitory effect of the female sex hormone on tumors, as observed in their experiments, was unrelated to the general nutritional state of the animals.

TABLE II: MEAN BODY WEIGHT CHANGES OF RATS RECEIVING 14 INJECTIONS OF ESTROGEN IN 52 TO 54 DAYS AS COMPARED WITH UNTREATED ANIMALS

	No. rats	Sex	Mean weight at onset	Mean weight at end	Difference
Control	13	♀	139 ± 2.1	163 ± 2.8	+ 24
	13	♂	192 ± 5.6	217 ± 5.8	+ 25
Treated	19	♀	145 ± 1.2	143 ± 1.5	— 2
	14	♂	184 ± 2.2	173 ± 1.8	— 11

Caloric restriction and tumor growth.—As inhibition in the growth of the transplanted mammary cancer of the rat (R 2426) in animals subjected to prolonged estrogen treatment was associated with a tendency to loss in body weight, it was obviously necessary to investigate the rate of tumor growth in animals in which loss of weight was deliberately produced by another means. The simplest method is direct restriction of the caloric intake. Bischoff, Long, and Maxwell (1, 2) have reported that a 50 per cent reduction in caloric intake results in retardation in growth of sarcoma 180 in mice. In utilizing the rat mammary cancer for an experiment of this type a serious difficulty is the slow rate of proliferation of the tumor. It is necessary to maintain the severe caloric restriction for the same period employed for prolonged estrogen administration. The mortality among underfed rats is frequently high. Table III compares the body weight changes in animals given an adequate caloric intake (the prepared mixture of fabricated foods previously described) and rats fed approximately 50 per cent of this quantity. In one-half the animals the dietary control was instituted 14 days prior to tumor implantation, but the result did not differ from that observed in the remaining rats in which food restriction was begun simultaneously with transplantation. In Table III are also recorded the mean tumor diameters of R 2426, 46 to 48 days after transplantation in control and underfed groups, the females and males being considered together as the results in both sexes were comparable. The mean tumor diameter in the underfed group is significantly less than in adequately nourished animals. The degree of tumor inhibition does not differ strikingly from that produced by excessive female sex hormone. Nevertheless the extent of loss of weight produced by drastic dietary restriction did exceed that produced by estrogen. As the hormone produced characteristic morphologic alterations in the transplanted adenocarcinoma, to be described in a succeeding section, it cannot be excluded that some retardation in tumor growth resulted from a specific action of the hormone.

TABLE III: MEAN BODY WEIGHT CHANGES OF RATS ON REDUCED FOOD INTAKE AS COMPARED WITH ANIMALS ON ADEQUATE DIET, AND MEAN TUMOR DIAMETERS (R 2426) IN THESE RATS, 46 TO 48 DAYS AFTER TRANSPLANTATION

	No. rats	Sex	Mean daily intake	Mean weight at onset	Mean weight at end	Difference
I. Adequate diet	20	♀	11-12 gm.	128 ± 3.5	157 ± 2.3	+ 29
	18	♂	13-16 gm.	175 ± 3.0	213 ± 2.7	+ 38
II. Underfed	22	♀	5-6.5 gm.	143 ± 1.6	117 ± 1.8	— 26
	16	♂	7-8 gm.	185 ± 3.7	159 ± 3.9	— 26

	Tumor diameter in mm., mean ± P.E.	Difference ± P.E.	Difference / P.E. difference
I	22.8 ± 0.47	..	..
II	14.7 ± 0.54	8.1 ± 0.72	11.2

Estrogen in rats bearing transplanted sarcoma.—In order to test the effect of the female sex hormone on a tumor composed of mesodermal elements, 12 rats (6 females, 6 males) bearing transplantable sarcoma IRS 4337 were given injections of estrogen according to the previously adopted plan. This tumor, a spindle cell sarcoma described by Dunning, Curtis, and Bullock (5), arose in the liver of a rat of the inbred Copenhagen strain as a result of *Cysticercus* infestation. In contrast to the transplantable adenocarcinoma (R 2426), it grows rapidly. Estrogenized animals bearing sarcoma IRS 4337 showed the general effects of treatment, but no tumor inhibition occurred. The experiment was terminated 22 days after transplantation when rats which received 8 injections of estrogen

(2 doses prior to and 6 following implantation of the tumor) had large tumors comparable with control growths. Beginning ulceration of some tumors necessitated termination of the experiment at this stage.

No tumor inhibition occurred in a group of Fischer rats (9 females, 7 males) bearing a second transplantable sarcoma, IRS 1548, and to which similar quantities of estrogen were administered. The experiment was terminated after 25 days, as many treated and control rats had ulcerated tumors. This tumor, a polymorphous cell sarcoma, was described by Bullock and Curtis (4), and arose subcutaneously from a *Cysticercus* larva transplanted from the liver.

Secretory changes in transplanted adenocarcinoma of the breast as a result of estrogen treatment.— Estrogen stimulates the growth of the normal mammary gland, and the resulting mammary hyperplasia and secretory phenomena probably occur through stimulation of a pituitary lactogenic hormone. The latter hormone has been isolated in both sexes.

The possibility of functional activity by benign and malignant tumors is well known. It is sufficient to recall such growths as keratinizing squamous cell cancer, osteogenic sarcoma, and hormone-secreting tumors of the thyroid, parathyroid, gonads, and adrenal glands in females and males.

The production of secretory changes in existing malignant epithelial tumors by hormones which act as physiologic stimulants of their tissue of origin is, however, an unusual occurrence. Estrogen, as employed in the present experiments, produced lactational changes in the transplanted mammary cancers. The sex of the tumor-bearer exerted no determining influence in the production of these effects. It is appropriate to stress that active lactation phenomena were never observed in tumors of untreated rats. The changes induced by estrogen were focal and distributed at random throughout the tumors. In early stages the secretory phenomena occurred in small zones evident only on microscopic examination. Secretory vacuoles appeared in the cells of a limited number of tumor acini, but the predominant mass of the growth remained unaffected. Some alveoli contained both normal and secreting tumor cells. In later stages, at a time generally corresponding with the injection of considerable quantities of hormone, the tumors to a large degree had the appearance of cystic masses containing thick milk-like fluid. The functioning alveoli were lined by one or several layers of cells containing large secretory fat droplets which flattened the nuclei. These droplets were situated in the portion of the cells bordering the acinar cavity or in the basal zones. As a consequence of the initiation of functional activity the affected acini became more regular and rounded in shape. The number and size of the papillary formations, as seen frequently in normal tumors, regressed, and as secretory changes progressed, cystic formations developed. The cells lining cystic secreting tumors were flattened. The small and dilated cavities of lactating alveoli contained secretion enclosing fat droplets and occasionally fragments of detached portions of cells. Thus, the apocrine type of secretion, known to occur in the normal functioning mammary gland, was reproduced. The variable state of activity of the lactating tumors again reproduces the normal lactating mammary gland which also fluctuates in its degree of response to the factors stimulating secretory function.

Mitotic figures, which occur with moderate frequency in untreated growths, were either completely absent or encountered only in rare instances in functioning tumor tissue. Thus, the rate of cell proliferation in functioning zones is undoubtedly diminished. It is tempting to speculate on the possibility of an adequate hormonal stimulation producing a progressive restoration of a malignant tumor to functioning tissue at the ultimately complete expense of its proliferative activity. Certainly when lactation in the tumors of estrogenized animals was advanced, their histologic structure was sufficiently altered to make it difficult to realize that this growth was formerly a solid mass of adenocarcinomatous tissue. The grafted tumors in estrogen-treated rats resembled more closely normal lactating breast or secreting adenoma than a malignant epithelial growth. The microscopic features of the progressive lactational changes in the tumors are illustrated in Figs. 2, 3, 4, and 5.

As seen in Table IV the majority of estrogen-treated rats that survived longer than 21 days after tumor transplantation had lactating growths. This was the minimal time necessary for the production of secretory changes occurring in a female rat that received 8 injections. The percentage of lactating tumors was undoubtedly higher than indicated in Table IV. In evaluating the changes histologically only one to three sections of the tumors were made, and it is probable that a study of serial sections of apparently negative tumors would have revealed functioning zones. Further, a number of growths are omitted which occurred in animals that succumbed during the period in which secretory changes were observed in the grafts in other animals. Unfortunately sections of these tumors were not prepared.

In an attempt to estimate more exactly the minimal amount of estrogenic hormone required to produce these alterations in the tumor, injections were administered to 10 female and 9 male rats beginning 14 days after tumor transplantation and continued twice weekly. The first tumors were removed from both sexes after 3 days and additional tumors were re-

TABLE IV: INCIDENCE OF OCCURRENCE OF LACTATING CHANGES IN TRANSPLANTABLE CARCINOMA OF THE RAT BREAST (R 2426) FOLLOWING ESTROGEN INJECTIONS

Females	No. rats	23
Tumors with secretory changes	19	Tumors 21-75 days, 8-21 injections
Unchanged	4	Tumors 59-75 days, 18-21 injections
Males	No. rats	18
Tumors with secretory changes	13	Tumors 32-84 days, 12-21 injections
Unchanged	5	Tumors 21-73 days, 8-21 injections
Total	No. rats	41
Tumors with secretory changes	32(78%)	
Unchanged	9	

Transplantation of tumors of estrogenized rats.—One tumor which had been growing for 64 days in a female rat which received 19 injections of estrogen was transplanted into 17 untreated rats. The proliferative rate of this tumor had been reduced, but when transplanted it contained no gross or microscopic lactating zones. The tumors ensuing from these implants showed no differences in growth rate or structure from tumors arising from implants in untreated animals. Tissue of 3 tumors in which milk production dominated grossly was transplanted in 26 animals; 7 females received implants of 2 tumors on each side of the body and 19 animals (9 females, 10 males) single fragments of one tumor. In this group a trend to an increase in the latent period of the resulting growths was evident. Following implantation of untreated tumors it is unusual for a palpable sign of a growing transplant to be delayed beyond 14 days. On the other hand, of the 33 lactating transplants growth of 10 was evident after 14 days, of 5 after 21 days, of 3 after 28 days, of 11 after 35 days, of 1 after 49 days, of 1 after 78 days, and of 1 after 106 days. One animal died after 108 days without evidence of tumor.

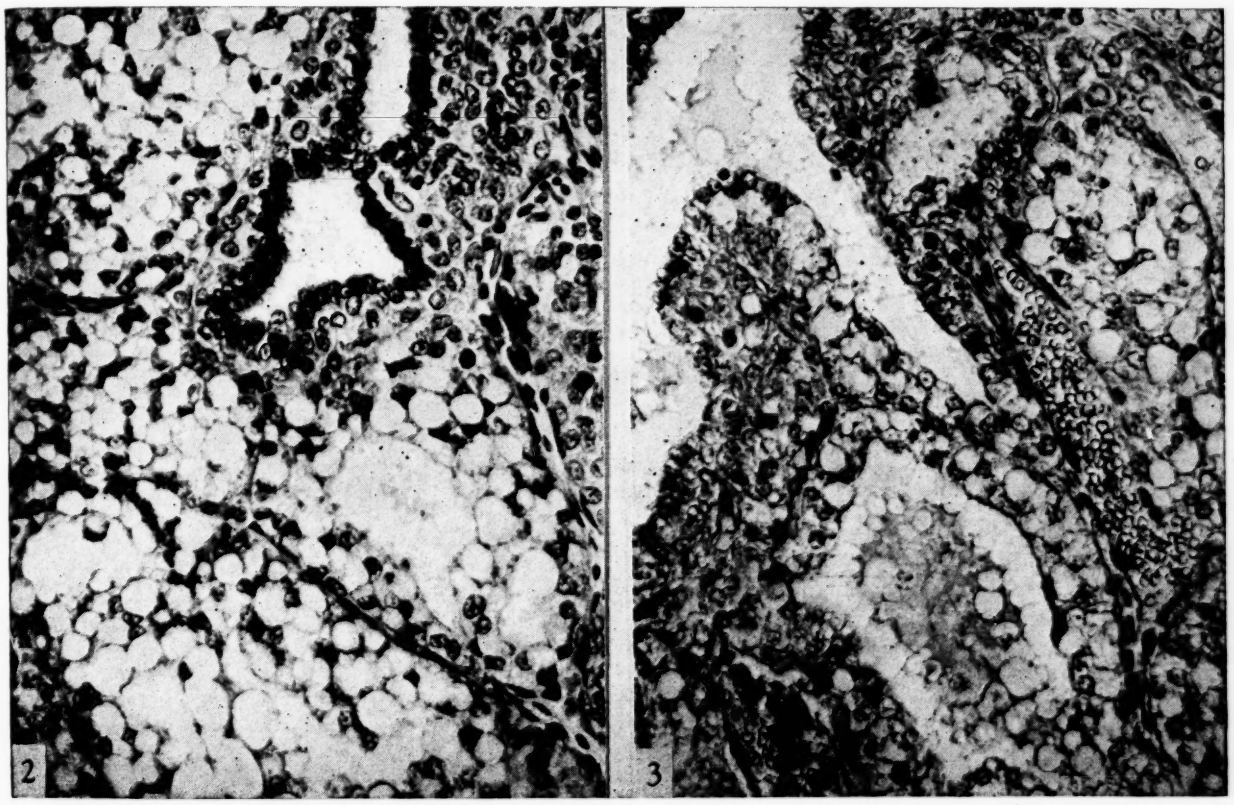


FIG. 2.—Focal zone of lactating transplantable carcinoma of the rat's breast (R 2426) bordering on unchanged tumor. Tumor 45 days, 14 injections of estrogen. Mag. × 375.

FIG. 3.—More extensive lactation phenomena in transplantable carcinoma of the rat's breast (R 2426). Beginning cystic changes are visible in tumor acini in which are accumulated secretion consisting of fat droplets and fragments of detached cells. Tumor 52 days, 17 injections of estrogen. Mag. × 375.

moved after each dose of hormone. In this group, however, the earliest secretory phenomena were not observed until after 50 and 52 days in female and male hosts that received 11 injections of hormone. An unusual finding in this group was a pulmonary metastasis with focal lactating zones in a female rat which had received 18 injections of estrogen during a period of 77 days after implantation of the tumor (Fig. 6).

The tumors arising in lactating fragments reproduced the morphology of the original untreated tumor. The delay in growth of the lactating transplants may be the combined result of the inoculation of the relatively small number of cells present in the cystic milk-containing zones and the large number of cells in a functioning state. The ultimate fate of these secreting cells is unknown. The tumors are perhaps produced by the persisting nonfunctioning cells. No method has been devised to ascertain whether transplants of totally lactating tumor tissue would grow progressively.

Tumors during pregnancy and lactation.—Following the demonstration that excessive female sex hormone was capable of giving rise to functional activity in the transplanted adenocarcinoma it was of interest to investigate the influence of pregnancy and lactation. Heiman and Krehbiel (12) observed milk production in transplanted mammary fibroadenoma of the rat during pregnancy.

Data for 6 animals are available on the association of pregnancy and lactation with the transplanted breast cancer. In 4 animals the young were born 6, 13, 15, and 31 days after tumor transplantation and nursed for 2 to 5 weeks. One animal, inoculated 2 days after littering, also nursed her young for 3 weeks and 1 tumor-bearer died during the latter half of pregnancy. In nursing animals the tumors were examined at the conclusion of lactation. There was no discernible alteration in the growth or structure of the tumors in this series of rats.

Testosterone and tumors.—The effect of testosterone propionate [1] was tested in 12 animals (6 females, 6 males). In 6 rats 2 injections of 2.0 mgm. each of the androgenic hormone dissolved in 0.1 cc. sesame oil were given in the week prior to transplantation and 20 treatments in the succeeding period. Six animals received 6 injections of similar quantities beginning 14 days after tumor implantation. In no case was an effect observed on the growth rate or structure of the tumor.

Castration.—Tumor fragments were transplanted in 8 adult females and 10 males castrated the previous day. The tumors in this series were comparable with those in control rats.

Summary and Discussion

Subcutaneous injections of estrogen in doses of 0.166 to 0.2 mgm. in sesame oil twice weekly in the inbred August strain of rats bearing a regularly growing transplantable adenocarcinoma of the breast inhibited tumor growth. Tumors in treated rats were significantly smaller than in control animals. The growth rate of tumors in treated as in untreated rats is wholly independent of the age or sex of the animals. Estrogenized animals in general showed a loss of body weight. Reduction in weight produced by a caloric restriction of approximately 50 per cent was also associated with a decrease in size of transplanted tumors. The growth rate of two rapidly proliferating transplanted sarcomas in inbred rat strains was not inhibited by estrogen, despite loss of weight in the treated animals.

Lactational changes in the transplanted mammary cancers were produced in a large majority of female and male rats that survived 21 days after implantation of grafts and received 8 doses of estrogen. This was the minimal quantity of female sex hormone required to produce active secretory changes in the tumors. The lactational phenomena varied in extent in different tumors. Some contained microscopic focal areas of secretion distributed at random, while others were extensively transformed into large cystic spaces containing milk-like fluid. In one treated animal a lactating metastasis in the lung was observed.

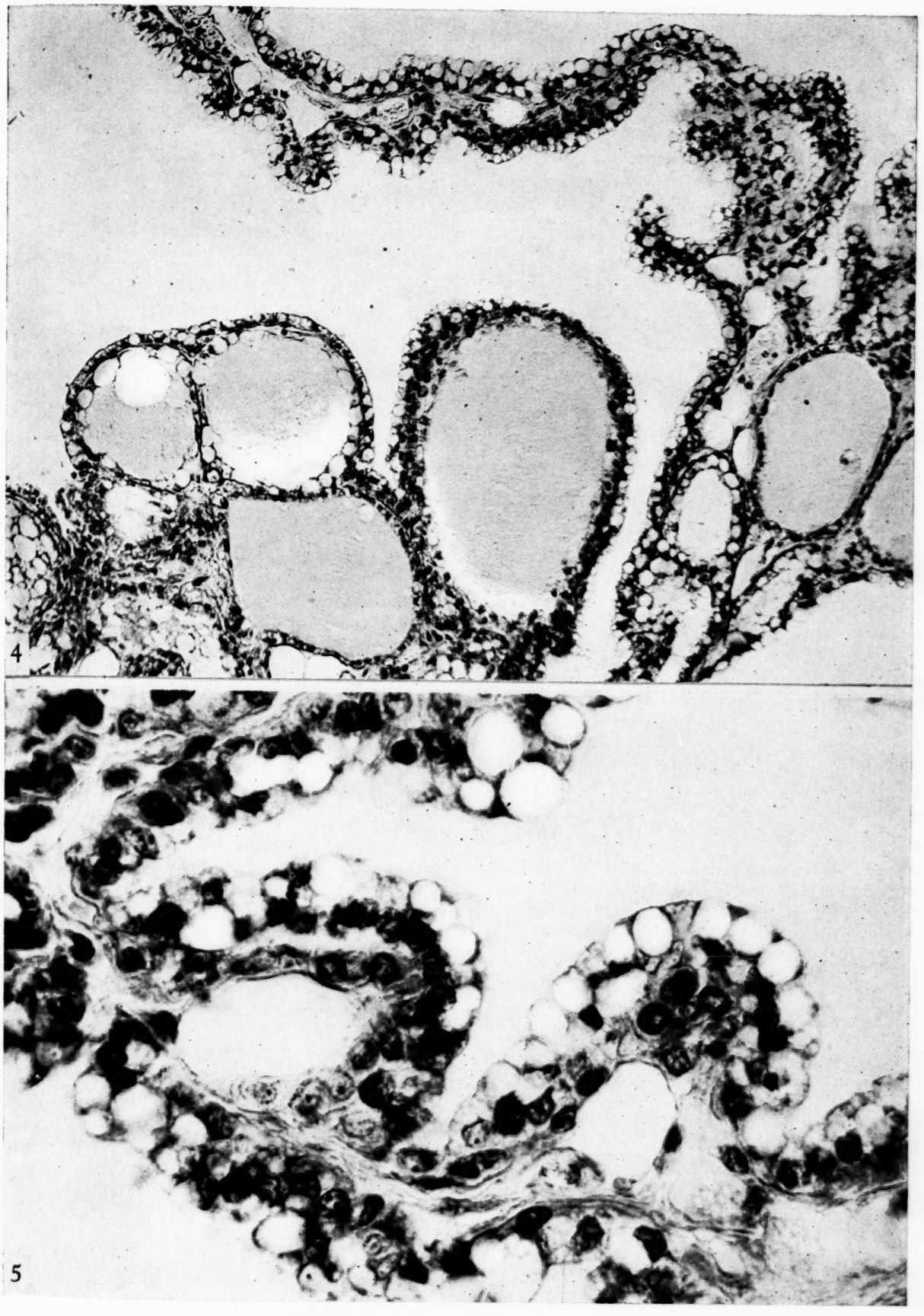
It cannot be ruled out that some degree of inhibition of tumor growth resulted from the transformation of potentially proliferating tumor into functioning tissue as a result of adequate hormonal stimulation. The growth and morphology of benign mammary tumors under certain circumstances are dependent on variations in the normal hormone balance. A benign neoplasm, therefore, may differ only quantitatively from the malignant form in its aberration from normal tissue, as a malignant tumor in the present experiments proved capable of radical alteration by an excessive supply of the specific hormone which acts physiologically as a stimulus of its tissue of origin.

No alteration was observed in the growth rate of transplanted tumors arising from implants of an estrogenized tumor of diminished rate of proliferation,

DESCRIPTION OF FIGURES 4 AND 5

Fig. 4.—Advanced lactation changes in transplantable carcinoma of the rat's breast (R 2426). The tumor is transformed into a conglomerate of cystic cavities lined by secreting cells, with no vestige of the original solid tumor structure apparent. Tumor 75 days, 21 injections of estrogen. Mag. × 187.5.

Fig. 5.—High power enlargement of a zone of the tumor illustrated in Fig. 4. Mag. × 875.



FIGS. 4 AND 5

but without evidence of secretion. The latent period of tumors originating from transplants of grossly lactating tumor tissue was considerably prolonged. In both instances the ensuing tumors reproduced the structure of untreated growths.

Pregnancy and lactation, testosterone propionate, and castration exerted no demonstrable influence on the growth or structure of the mammary adenocarcinoma.

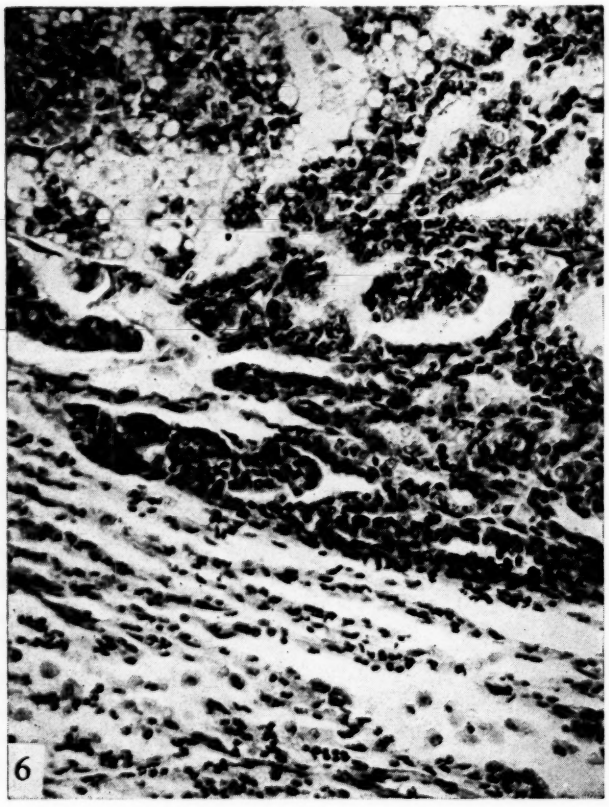


FIG. 6.—Pulmonary metastasis of transplantable carcinoma of the rat's breast (R 2426) in an animal with a subcutaneous tumor of 77 days which had received 18 injections of estrogen. Note the focal area of lactating tumor in the upper left corner. Mag. × 187.5.

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The Inhibition of Phospholipid Oxidation by Carcinogenic and Related Compounds*†

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The biochemical mechanism for the production of tumors by the carcinogenic hydrocarbons is still unknown. This is in part due to the experimental difficulty involved in studying the effect of these substances *in vitro,* for they are soluble in fat and insoluble in water, whereas the enzyme systems usually considered as counterparts of cellular reactions are composed of water-soluble materials. Since an intimate contact between reactants is a necessary prerequisite for any reaction, it is not surprising that water-insoluble hydrocarbons have failed to alter aqueous systems. On the other hand, systems containing fat-soluble members might more reasonably be expected to respond to the hydrocarbons. An example of a system applicable to such studies is the oxidation of phospholipids in the presence of buffered aqueous solutions of glutathione, first described by Hopkins in 1925 (10). These emulsions constitute a simplified but reasonably close model of the colloidal mechanism by which fat-soluble materials are brought into contact with aqueous catalysts in the cell. The present studies deal with the oxidation of phospholipids catalyzed not only by glutathione but by other substances which have not previously been demonstrated to induce oxidation. The effect of hydrocarbons and related compounds on such systems is described.

Methods

The experiments were performed in an ordinary Warburg manometric apparatus. Emulsions of phospholipids in buffer were treated with solutions of catalyst, and after equilibration at 38.2° C., the rate of oxygen consumption was determined. The total volume of the solution was 2.0 cc., and in addition, 0.2 cc. of 20 per cent KOH were added to bits of filter paper in the inset cup of the flask. The flasks were shaken 60 times a minute at an amplitude of 3 cm., and the experiments usually continued for 4 hours. The buffers used were M/20 potassium biphthalate-HCl at pH 3.5, or M/40 Sörensen phosphate buffers at pH 6.8 or 7.4.

The following biochemical substances were tested for catalysis of phospholipid oxidation: glutathione, cysteine, glutamic acid, glycine, ascorbic acid, thiamin, riboflavin, pyridoxine, benzoyl peroxide, alloxan, sodium lactate, urea, alpha tocopherol, and 2-methyl-1,4-naphthoquinone. All were crystalline materials obtained commercially. Besides these, partially purified preparations of succinic dehydrogenase, coenzyme I, nucleic acid, cytochrome, and adenylic acid were used. To study the modifying effects of other compounds, the basic system usually contained ascorbic acid or glutathione as the catalyst plus buffer and phospholipid. The "modifiers" consisted of carcinogenic hydrocarbons, related noncarcinogenic chemicals, azo dyes, hormones, or anti-oxidants. These were all crystalline compounds obtained commercially. The specific compounds and the dosages are listed in Tables I to VII.

The phospholipids were obtained from egg yolk or from rat liver. Egg phospholipid was prepared by diluting the yolks with an equal volume of 10 per cent NaCl and shaking the mixture repeatedly with ether. Ten grams of anhydrous sodium sulfate were added to each 100 cc. of ether and the latter decanted and concentrated to a small volume under reduced pressure. The phospholipid was precipitated by adding 4 to 5 volumes of acetone and was reprecipitated from ether once or twice more. Phospholipid was prepared from fresh rat liver by covering the liver with 2 volumes of acetone, mincing in a Waring mixer, centrifuging, and re-extracting with a 1:4 alcohol-ether mixture, the solvent evaporated under reduced pressure, and the residue taken up in a small volume of ether and poured into acetone. The phospholipid was taken up in ether and reprecipitated 3 to 5 times. All preparative procedures were carried out as rapidly as possible and exposure to air reduced to a minimum. The ether used was peroxide-free. The final products were either stored under acetone for a few days, or they were dried *in vacuo* and sealed in evacuated tubes.

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For incorporation into the buffers used in the manometric experiments, the phospholipids were dissolved in ether and added to the calculated amount of buffer in a small Erlenmeyer flask. Suction was then applied, and the ether removed at 38° C. with vigorous, continued shaking. The resulting aqueous suspensions were uniform and opalescent. In order to get the hydrocarbons and other water-insoluble compounds into the reaction mixture, known amounts were dissolved in ether, mixed thoroughly with an ether solution of phospholipid, and then added to the buffer as just described. When attempts were made to add the hydrocarbons to the phospholipid after the latter had been dissolved in water, extremely variable results were obtained. The various combinations of buffer, catalyst, phospholipid, and hydrocarbon studied are indicated in the tables of results. Most experiments were run at a pH of 3.5 or 7.4 and the mixtures contained 3 mgm. of phospholipid and 0.5 mgm. of catalyst per flask plus from 50 to 200 μgm. of hydrocarbon. At least three determinations were made of each combination, and some were repeated as often as ten times. The results given are averages.

Results

Catalysis of phospholipid oxidation.—In confirmation of others (10, 14, 17) we found that the oxidation of phospholipid was catalyzed by glutathione or cysteine (Table I). Glutathione was appreciably more active than cysteine, in spite of the fact that it contains fewer sulfhydryl groups per mgm. Catalysis is believed to be due to this grouping (10, 14). Thus on a molar basis, the superiority of glutathione was even more marked than is indicated by the figures based on weight. Glutamic acid and glycine were without effect on the phospholipid.

The results of varying the levels of both phospholipid and glutathione are given in Table II. While the total oxidation was greater with the higher levels of phospholipid, the uptake per mgm. after 4 hours was highest when only 1 mgm. of phospholipid was present per flask. Under these conditions oxidation was practically complete in 4 hours as shown by the plateau of the curve of oxygen uptake whereas with higher levels of phospholipid, oxidation was incomplete and still progressing at a fast rate at the end of the 4-hour period. When oxidation was allowed to proceed over a long enough period, the uptake per mgm. was the same at all levels of phospholipid. No essential difference in oxygen consumption was noted whether 0.5, 1, or 2 mgm. of glutathione were used as catalyst. Hence smaller amounts of both phospholipid and catalyst than those reported by other workers (10, 17) were used in our subsequent experiments.

Other substances tested and found to promote the oxidation of phospholipid were ascorbic acid, thiamin, riboflavin, pyridoxine, and methylene blue (Table III). Ascorbic acid gave the best oxidation at pH 3.5, being superior even to glutathione. Thiamin, riboflavin, pyridoxine, and methylene blue all catalyzed the oxidation of egg phospholipids at pH 6.8 or 7.4 but not at pH 3.5. Oxidation was of the same order of magnitude as that obtained with glutathione and egg phospholipid at pH 3.5. With rat liver phospholipids, however, oxidation in the presence of the B vitamins was approximately 20 to 30 cu. mm. oxygen less than when preparations from egg were used. With ascorbic acid the oxygen uptake of phospholipid from rat liver was 2 to 3 times greater than that obtained with preparations from egg yolks. An average run with 3 mgm. egg phospholipid in the presence of 0.5 mgm. ascorbic acid was 87 cu. mm. O_2 at the end of 4 hours compared to 192 cu. mm. O_2 with preparations from rat liver. Qualitatively similar differences between phospholipids were observed when glutathione was used as catalyst, 70 to 80 cu. mm. O_2 being consumed by egg phospholipid as compared to 90 to 100 cu. mm. O_2 by the liver phospholipids. A variety of substances tested and found to have no effect as pro-oxidants are listed in Table IV. While not catalyzing oxidation in itself,

Table I: Comparison of Glutathione and Its Constituents on Phospholipid Oxidation (3 Mgm. Egg Phospholipid at pH 3.5)

Catalyst	Amount, mgm.	O_2 uptake 4 hrs. cu. mm.
None	..	0
Glutathione	0.5	76
Glutathione	1.0	88
Cysteine	0.5	52
Cysteine	1.0	62
Cysteine	2.0	75
Glutamic acid	0.5	0
Glycine	0.5	0

Table II: Oxygen Uptake at Varying Concentrations of Glutathione and Egg Phospholipid (pH 3.5)

Glutathione	Phospholipid	O_2 uptake 4 hrs. cu. mm./mgm. phospholipid
0.5	1.0	31
1.0	1.0	44
2.0	1.0	38
0.5	2.5	26
1.0	2.5	29
2.0	2.5	33
0.5	5.0	19
1.0	5.0	22
2.0	5.0	22
0.5	10.0	11
1.0	10.0	13
2.0	10.0	11

benzoyl peroxide stimulated oxygen uptake in the presence of ascorbic acid or glutathione.

The mechanism by which glutathione promotes fatty acid oxidation is not clearly understood, although Meyerhof (14) has postulated the formation of a peroxide by two thiol groups, which then decomposes in such a way that oxygen is transferred to the unsaturated linkage of the fatty acid, while the original S-H groups are reconstituted. Thus the thiol group would be concerned essentially with oxygen transport.

TABLE III: CATALYSTS OF PHOSPHOLIPID OXIDATION

Catalyst	Amount mgm.	Phospholipid	Amount mgm.	pH	O_2 uptake 4 hrs. cu. mm.
Ascorbic acid	0.5	Egg	3.0	3.5	87
Ascorbic acid	0.5	Liver	3.0	3.5	192
Ascorbic acid	0.5	....	..	3.5	10
Thiamin	0.5	Egg	3.0	7.4	65
Thiamin	0.5	....	..	7.4	0
Riboflavin	0.5	Egg	3.0	7.4	72
Riboflavin	0.5	....	..	7.4	4
Pyridoxine	0.5	Egg	3.0	7.4	63
Pyridoxine	0.5	....	..	7.4	3
Methylene blue	0.1	Egg	3.0	7.4	60
None	..	Egg	3.0	7.4	2
None	..	Egg	3.0	3.5	7
None	..	Liver	3.0	3.5	10

TABLE IV: SUBSTANCES WHICH DID NOT CATALYZE PHOSPHOLIPID OXIDATION

Substance	Amount mgm.	Phospholipid 3 mgm.	pH	O_2 uptake 4 hrs. cu. mm.
Glycine	1.0	Egg	3.5	0
Glutamic acid	1.0	Egg	3.5	0
Benzoyl peroxide	0.1	Egg	3.5	0
Alloxan	M/100	Egg	3.5	0
$CuSO_4$	0.3	Egg	3.5	0
Nucleic acid	1.0	Liver	3.5	0
Muscle mince	10.0	Liver	3.5	0
Cytochrome	0.1 cc. 10^{-7} M	Liver	3.5	15
Urea	0.5	Liver	3.5	10
α-Tocopherol	0.5	Liver	3.5	0
2-methyl-1,4-naphthoquinone	0.5	Liver	3.5	0
Adenylic acid	0.1	Liver	7.4	0
Succinic dehydrogenase (extract)	..	Egg	7.4	15
Na lactate	M/300	Egg	7.4	0
Coenzyme I (extract)	..	Egg	7.4	0

Riboflavin is known to be a component of enzyme systems which catalyze hydrogen transport while thiamin, as a part of co-carboxylase, is essential in carbohydrate metabolism. Neither uncombined vitamin, however, has hitherto been shown to exert any very obvious effect in oxidizable systems *in vitro*. The role of ascorbic acid and of pyridoxine in the cell is still obscure. The fact, however, that these substances exert a pronounced effect on phospholipid oxidation *in vitro*, might suggest a connection between these vitamins and phospholipid metabolism *in vivo*. The recent report of Mannering *et al.* (13) is an example of such a relationship. These authors demonstrated that increasing the level of fat in a riboflavin low ration had a corresponding deleterious effect on the growth of young rats. When riboflavin was administered, the rats were able to metabolize the fat.

Inhibition of phospholipid oxidation with carcinogenic chemicals.—The effect of certain carcinogenic chemicals on the catalyzed oxidation of phospholipids is given in Table V. The most effective inhibitors were p-dimethylaminoazobenzene and 2-amino-5-azotoluene which, at 100 μgm. per flask, decreased oxidation of liver phospholipid by 91 per cent and 83 per cent respectively. At 50 μgm. level the inhibition was 82 and 78 per cent. Of the carcinogenic hydrocarbons, the most active inhibitor was benzpyrene. When 100 μgm. per flask were added to liver phospholipid and ascorbic acid, an average of 81 per cent reduction of oxygen uptake was observed, compared to 64, 42, and 25 per cent inhibition when 9,10-dimethyl-1,2-benzanthracene, methylcholanthrene, and 1,2,5,6-dibenzanthracene were employed respectively. The estrogenic hormones dihydroestrone and estrone gave 76 per cent and 42 per cent inhibition respectively when added to liver phospholipid and ascorbic acid. In contrast to the hydrocarbons themselves their water-soluble derivatives, methylcholanthrene-9,10-endo-α,β-succinate, and 1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate were without activity.

The inhibition of phospholipid oxidation with the carcinogens was also observed when catalysts other than ascorbic acid were used. Benzpyrene added at 100 μgm. per flask to egg phospholipid inhibited the oxidation catalyzed by glutathione, cysteine, thiamin, riboflavin, pyridoxine, and methylene blue. When the latter four substances were used the reaction was carried out at pH 7.4. Furthermore, the addition of certain other substances to the phospholipid mixture did not interfere with the inhibiting action of benzpyrene. Thus, when 100 μgm. of benzoyl peroxide were added to egg phospholipid and glutathione, the oxygen uptake in a 4-hour period was increased from 86 cu. mm. to 248 cu. mm. The addition of 100 μgm. of benzpyrene to this latter combination, however, kept the total oxidation in the same period to only 20 cu. mm. Similarly the addition of $CuSO_4$, maleic acid, or alloxan to the phospholipid hydrocarbon mixture did not prevent the inhibition by the carcinogen.

Since only a small fraction of the known carcinogens were used in this study, accurate correlations cannot be made, but within the group tested, the degree of inhibition tended to vary with the carcinogenic potency. Thus, less inhibition was obtained with 1,2,5,6-dibenzanthracene than with the other more potent carcinogens. However methylcholanthrene is somewhat more potent as a carcinogen than benzpy-

rene although the inhibition in phospholipid oxidation was less than that obtained with benzpyrene. The most rapidly acting carcinogen on mouse (4) and rabbit skin [1] is 9,10-dimethyl-1,2-benzanthracene and it also was an effective inhibitor of phospholipid oxidation, although again, the degree of inhibition was not as high as that obtained with benzpyrene. However, 9,10-dimethyl-1,2-benzanthracene is not as good a carcinogen as benzpyrene when injected subcutaneously into mice (4). Little or no inhibition of phospholipid oxidation was obtained when the water soluble derivatives methylcholanthrene-9,10-endo-α,β-succinate or 1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate were used. It has been suggested that these compounds are carcinogenic only if changed back into the original hydrocarbons by the tissues. Dibenzanthracene-9,10-endo-α,β-succinate will hydrolyze in contact with tissues (4) and it has been shown to be a good carcinogen whereas methylcholanthrene-9,10-endo-α,β-succinate, which is stable, does not produce tumors when injected subcutaneously into mice.[1] Perhaps the lack of inhibition on phospholipid oxidation displayed by these two compounds is related to their stability under the conditions of the experiment.

In the presence of liver phospholipid and ascorbic acid, the azo dyes inhibited oxidation more effectively than any of the hydrocarbons. Here, too, there was some indication that the greater inhibition was obtained with the most rapidly acting carcinogen. Butter yellow (p-dimethylaminoazobenzene) inhibited phospholipid oxidation to a greater extent than aminoazotoluene, and in the rat it is also a more effective carcinogen. The high degree of inhibition observed when the azo dyes were used is in harmony with the fact that they are effective in producing liver tumors while the hydrocarbons are not. This parallelism between the experiments *in vitro* and *in vivo* was particularly close since the phospholipid used was prepared from rat liver, a tissue which is peculiarly sensitive to tumor production with the azo dyes and which incidentally is also high in ascorbic acid. The estrogens were also potent inhibitors of phospholipid oxidation, dihydroestrone being more effective than

TABLE V: INHIBITION OF PHOSPHOLIPID OXIDATION WITH CARCINOGENS (3 MGM. PHOSPHOLIPID AND 0.5 MGM. CATALYST PER FLASK, pH 3.5)

Carcinogen	Catalyst	Phospholipid	Per cent inhibition with various substances: 100γ Range	100γ Average	200γ Range	200γ Average
Benzpyrene	Ascorbic acid	Liver	69-93	81	91-92	92
Benzpyrene	GSH *	Egg	72-100	93	....	..
Benzpyrene	GSH * and glutamic acid †	Egg	87-97	92	....	..
Benzpyrene	Cysteine	Egg	40-74	57	....	..
Benzpyrene	Cysteine and glutamic acid †	Egg	65-83	74	....	..
Benzpyrene	Thiamin §	Egg	82-94	88	....	..
Benzpyrene	Riboflavin §	Egg	85-93	89	....	..
Benzpyrene	Pyridoxine §	Egg	87-91	89	....	..
Benzpyrene	Methylene blue ‡ §	Egg	75-81	78	....	..
Benzpyrene	GSH *+$CuSO_4$, 400γ	Egg	25-47	36	....	..
Benzpyrene	GSH *+maleic acid M/100	Egg	80-84	82	....	..
Benzpyrene	GSH *+alloxan M/100	Egg	35-59	47	....	..
Benzpyrene	GSH *+benzoyl peroxide 100γ	Egg	60-74	67	....	..
9,10-Dimethyl-1,2-benzanthracene	Ascorbic acid	Liver	63-65	64	67-88	78
Methylcholanthrene	GSH *	Egg	70-85	78	....	..
Methylcholanthrene	Ascorbic acid	Liver	42-43	42	60-73	67
1,2,5,6-Dibenzanthrene	GSH *	Egg	45-88	52	....	..
1,2,5,6-Dibenzanthrene	Ascorbic acid	Liver	19-30	25	56-71	64
p-Dimethylaminoazobenzene	Ascorbic acid	Liver	89-94	91	....	..
Aminoazotoluene	Ascorbic acid	Liver	82-83	83	....	..
Methylcholanthrene-9,10-endo-α,β-succinate	GSH *	Egg	0	0	....	..
Methylcholanthrene-9,10-endo-α,β-succinate	Ascorbic acid	Liver	2-5	4	4-5	5
1,2,5,6-Dibenzanthracene-9,10-endo-α,β-succinate	GSH *	Egg	0-0	0	....	..
1,2,5,6-Dibenzanthracene-9,10-endo-α,β-succinate	Ascorbic acid	Liver	0-6	3	4-12	8
Dihydroestrone	GSH *	Egg	90-95	92	....	..
Dihydroestrone	Ascorbic acid	Liver	68-80	76	82-87	85
Estrone	GSH *	Egg	60-68	65	....	..
Estrone	Ascorbic acid	Liver	40-43	42	62-70	67

* Glutathione.
† 0.5 mgm. of each per flask.
‡ 0.1 mgm. per flask.
§ pH 7.4.

[1] Unpublished data.

estrone. Inhibition thus paralleled estrogenic potency. Opinion is still divided as to whether the estrogens should be considered true carcinogens since their cancer producing activity is indirect and limited to specific tissues, strains, and species of animals. Only a few subcutaneous tumors have been reported arising at the site of injection of these compounds (8).

Inhibition of phospholipid oxidation with noncarcinogens. That the inhibition of phospholipid oxidation was not confined to the carcinogens is shown in Table VI. The greatest inhibition was obtained with hydroquinone, catechol, dihydroxydiphenyl, and carotene, while next in order were phenanthrene, cholesterol, anthracene, desoxycholic acid, anthraquinone, benzanthraquinone, and 9,10-dimethyl-1,2-benzanthracene peroxide. The sex hormones, dehydroandrosterone and progesterone, inhibited only slightly compared with the carcinogenic estrogens. Alloxan gave definite inhibition of liver phospholipid-ascorbic acid oxidation whereas it actually stimulated oxygen uptake of egg lipid in the presence of glutathione (Table VII). Benzanthraquinone did not inhibit egg phospholipid oxidation with glutathione but acted as an anti-oxidant when ascorbic acid, thiamin, or methylene blue were used as catalysts. Anthraquinone inhibited oxygen uptake when thiamin, methylene blue, and glutathione were used.

The inhibition of the autoxidation of fats and oils by hydroquinone and catechol is well known and hence it is not surprising that these anti-oxidants should also inhibit the catalyzed oxidation of phospholipids. However, so far as we are aware, the role of anti-oxidant has not been ascribed to the other compounds tested. Carotene is actually a pro-oxidant under some conditions but it inhibited this reaction.

TABLE VI: INHIBITION OF PHOSPHOLIPID OXIDATION WITH NONCARCINOGENS (3 MGM. PHOSPHOLIPID AND 0.5 MGM. CATALYST PER FLASK, pH 3.5)

Compound tested	Catalyst	Phospholipid	Per cent inhibition with various amounts: 100γ Range	100γ Average	150γ Range	150γ Average	200γ Range	200γ Average
Hydroquinone	GSH *	Egg	95-100	98	....	..	....	..
Hydroquinone	Ascorbic acid	Liver	36-41	38	....	..	70-89	78
Catechol	GSH *	Egg	96-100	99	....	..	....	..
Catechol	Ascorbic acid	Liver	19-26	21	....	..	40-59	45
Dihydroxydiphenyl	GSH *	Egg	95-100	98	....	..	....	..
Dihydroxydiphenyl	Ascorbic acid	Liver	78-91	82	....	..	79-90	82
Carotene	Ascorbic acid	Liver	75-83	81	....	..	70-90	86
Phenanthrene	GSH *	Egg	20-25	22	13-32	25	....	..
Phenanthrene	Ascorbic acid	Liver	43-52	48	....	..	91-92	91
Cholesterol	GSH *	Egg	0-41	18	17-36	23	....	..
Cholesterol	Ascorbic acid	Liver	42-43	43	....	..	71-90	81
Anthracene	GSH *	Egg	19-33	24	10-33	26	....	..
Anthracene	Ascorbic acid	Liver	37-47	42	....	..	70-76	73
Desoxycholic acid	GSH *	Egg	0-20	10	0-23	15	....	..
Desoxycholic acid	Ascorbic acid	Liver	32-37	34	....	..	73-82	77
9,10-Dimethyl-1,2-benzanthracene peroxide	Ascorbic acid	Liver	30-41	38	....	..	45-50	49
Anthraquinone	GSH *	Egg	35-46	44	....	..	....	..
Anthraquinone	Thiamin ‡	Egg	....	10	....	..	....	..
Anthraquinone	Methylene blue †	Egg	....	23	....	..	....	..
Benzanthraquinone	GSH *	Egg	....	0	....	..	....	..
Benzanthraquinone	Ascorbic acid	Liver	24-26	25	....	..	33-42	37
Benzanthraquinone	Thiamin ‡	Egg	....	40	....	..	....	..
Benzanthraquinone	Methylene blue †	Egg	....	33	....	..	....	..
Dehydroandrosterone	GSH *	Egg	24-30	27	25-35	29	....	..
Dehydroandrosterone	Ascorbic acid	Liver	10-15	13	....	..	21-32	28
Progesterone	GSH *	Egg	....	..	20-32	27	....	..
Calciferol	GSH *	Egg	10-20	17	....	..	....	..
Alloxan	Ascorbic acid	Liver	20-32	28	....	..	34-45	40

* Glutathione.
† 0.1 mgm. per flask.
‡ pH 7.4.

Compounds which do not inhibit phospholipid oxidation.—Our experiments demonstrate that a considerable number of compounds inhibit the catalyzed oxidation of phospholipid. It must be remembered, however, that with most of these, the substances were chosen because of certain similarities. In Table VII are listed a few compounds whose presence in the system, with one variable exception, did not inhibit oxygen uptake. Phenol, glucose, cytochrome, iodoace-

tate, maleic acid, α-tocopherol, and 2-methyl-1,4-napthaquinone had little or no effect on the system. The effect with alloxan was variable depending upon the system used; *i.e.*, a slight but constant stimulation resulted with egg phospholipid and glutathione whereas a definite inhibition was noted when liver phospholipid and ascorbic acid were used. Benzoyl peroxide actually caused an increased oxygen uptake which in some runs was double the usual oxidation; in other words, there was a reversal of inhibition. This effect was best demonstrated using glutathione.

TABLE VII: COMPOUNDS WHICH DID NOT INHIBIT PHOSPHOLIPID OXIDATION (3 MGM. PHOSPHOLIPID AND 0.5 MGM. CATALYST PER FLASK, pH 3.5)

Added compound	Amount	Catalyst	Phospholipid	Oxygen uptake 4 hours, cu. mm. With compound	Oxygen uptake 4 hours, cu. mm. Without compound
Phenol	100γ	Ascorbic acid	Liver	148	156
Phenol	200γ	Ascorbic acid	Liver	144	156
Phenol	150γ	GSH *	Egg	82	86
Phenol	100γ	Methylene blue † ‡	Egg	58	61
Phenol	100γ	Thiamin †	Egg	59	65
Alloxan	150γ	GSH *	Egg	97	86
Benzoyl peroxide	150γ	GSH *	Egg	230	86
Benzoyl peroxide	100γ	Ascorbic acid	Liver	201	171
Glucose	200γ	Ascorbic acid	Liver	165	171
Cytochrome	0.1 cc 1×10^{-7} M	Ascorbic acid	Liver	167	171
Maleic acid	M/500	Ascorbic acid	Liver	169	171
Iodoacetate	M/500	Ascorbic acid	Liver	172	171
2-Methyl-1,4-naphthoquinone	200γ	Ascorbic acid	Liver	159	158
α-tocopherol	200γ	Ascorbic acid	Liver	156	158

* Glutathione.
† 0.1 mgm. per flask.
‡ pH 7.4.

DISCUSSION

The oxidation of phospholipids by several important cellular constituents *in vitro* suggests that such reaction may be involved in metabolic processes. However, relatively little is known about the exact metabolic function of either the phospholipids or of the catalysts employed in these experiments. This makes the interpretation of results doubly difficult. The fact that some of these oxidations occur only at a pH which is incompatible with physiological activity need not imply that a similar type of reaction cannot occur in the cell. All those compounds which were stable at a neutral pH also catalyzed oxidation at that pH. Thus, since ascorbic acid and glutathione exist in a reduced state *in vitro* only at an acid pH, it was necessary to carry out the experiments in an acid medium. Effective means of keeping these substances reduced at a neutral pH are present in the living cell. The action of the various vitamins in this reaction suggests a possible tie-up between fat and carbohydrate metabolism. There is considerable evidence that fat has a sparing action on vitamin B_1 and Longenecker *et al.* (12) have shown that fatty livers can be produced in rats on a fat-free diet by the administration of thiamin.

Various studies concerned with the biochemical mechanism of the carcinogenic compounds *in vitro* have been made. Boyland (2) demonstrated that oxidized carcinogenic hydrocarbons were more effective inhibitors of lactic dehydrogenase than are similar products from noncarcinogenic chemicals. He also observed that oxidized dibenzanthracene had an inhibitory effect on respiration and glycolysis of both normal and malignant tissues. The intact hydrocarbon, however, was without effect on the systems investigated. Various workers have reported on the photodynamic action of the carcinogens. Doniach (7) has shown that *Paramecia* were unaffected in a culture containing hydrocarbons if kept in the dark but, when light was applied, products were formed which were harmful to the organisms. Others have demonstrated similar results with cultures of yeast and tissues (9, 11). Still another approach has been concerned with the effect of the pure carcinogens on tissue cultures (5). Reported results vary depending on the method, type of hydrocarbon, and tissue used. An entirely different method of approach to the problem has been reported by Davis, Krahl, and Clowes (6). They found changes in the force-area curves of the surface films of cholesterol, dihydrocholesterol, and ergosterol in the presence of certain hydrocarbons. It is notable that these model experiments were composed of fat-soluble compounds.

Whether the inhibition of phospholipid oxidation by the carcinogenic hydrocarbons is of significance in the mechanism of cancer production, or whether the similar action of the estrogens is related to their physio-

logic action, is as yet unknown. Our present knowledge of the metabolism of the phospholipids or of any of the catalysts employed is still very meager. Furthermore, various noncarcinogenic chemicals also behaved as anti-oxidants. This does not, however, necessarily rule out the phenomenon as significant in carcinogenesis. There are no doubt many requirements necessary of any agent in order that it be able to induce cancer. One may be that it inhibits phospholipid oxidation; the failure of a carcinogen to inhibit phospholipid oxidation has not been observed.

The characteristics of a compound necessary for carcinogenic activity are undoubtedly complex. Some substances may possess one or more of such qualities but lack certain others required to initiate or to continue those reactions which eventually lead to cancer. Thus it is generally agreed that all carcinogenic agents must be present at a given site over a long period of time and in the proper concentration. Two exceptions to this rule are virus tumors and skin cancer in certain very susceptible mice when only 1 or 2 applications of hydrocarbon are applied (4). The amount of hydrocarbon that would enter the cell and remain there after such application is unknown, but Chalmers and Peacock (3) have shown that much of the chemical is rather quickly absorbed by the fat and excreted in a few days. However, even after 1 month the tissues showed strong fluorescence indicating that some hydrocarbon still remained bound in the cells. At all events, in all but this single painting experiment it has been necessary to inject a sufficient quantity at one time or to apply the carcinogen at frequent intervals in order to produce cancer. Water-soluble compounds such as catechol and hydroquinone, which inhibit the oxidation of phospholipids, would hardly be expected to possess carcinogenic activity since they could be so quickly removed from the tissues. If they were applied to the same site frequently, however, they might induce cancer. Even a glucose or HCl buffer mixture has been reported carcinogenic when injected frequently at the same site (15, 16). Furthermore, if the carcinogenic chemical is to remain in contact with the cell over a prolonged period it must not be too quickly metabolized or detoxified. The weaker carcinogenic potency of some of the estrogens may be explained by a more probable rapid rate of metabolic turnover than is found with the carcinogens.

Another prerequisite for carcinogenesis by *chemical* agents may well be solubility in fat. All of the patent carcinogens so far described are water-insoluble and fat-soluble. The water-soluble carcinogens, styryl 430, zinc chloride, and light green F.S. (sodium or calcium salts of diethyl-dibenzyl-diamino-triphenylcarbinol-trisulphonic acid) are much less active, possibly because they fail to persist in a certain area or maintain intimate contact with the necessary reactants to the same degree as the water-insoluble hydrocarbons.

A third factor to be considered is the degree of activity shown by a compound in a given reaction. It is possible that only partial rearrangement in certain cellular constituents or reactions is necessary for cancer production. Many agents are probably inactive as carcinogens because they induce changes which are either too feeble or too drastic. There may well be many properties besides those mentioned which are required of a chemical if it is to be carcinogenic, and even from our present knowledge, it appears that no one biological reaction, other than the end result itself, is sufficient to classify an agent as being carcinogenic. Hence it is not surprising that certain noncarcinogenic compounds should also possess some of the biochemical properties of the carcinogens. In this connection it is of interest to point out the parallelism existing between the inactivity of the succinic acid derivatives of dibenzanthracene and methylcholanthrene as carcinogens and their failure to inhibit phospholipid oxidation. This indicates that the 9,10 position is of importance for the inhibition of lipid oxidation as well as for carcinogenic activity.

It can probably be assumed that the carcinogenic hydrocarbons find entry into the cell by way of some fat-soluble substance. This leaves a variety of possibilities regarding the mode of action of the hydrocarbon. The physical properties of the lipids in the cell wall may be altered or there may be interference with the normal exchange of metabolites through this wall. It has been demonstrated that changes in surface films of cholesterol and ergosterol can be produced by various polycyclic hydrocarbons (6) and Bloor (1) has shown that phospholipids may act as oxygen carriers. Disturbances of these normal arrangements may lead to an altered cellular physiology. Once in the cell, the carcinogens may induce alterations in the lipids which in turn may be directly responsible for those changes necessary for cancer formation, or such changes may be accomplished indirectly through some other system. One possible alteration in fat metabolism is illustrated by the results of our experiments with phospholipid oxidation. Still another possibility is the alteration of the hydrocarbon into a water-soluble substance which in turn might initiate those reactions directly responsible for cancer. No experimental evidence exists, however, to support the latter assumption, but it would be interesting to determine whether a carcinogenic water-soluble substance can be extracted from tissues which have been treated with cancer producing hydrocarbons.

SUMMARY

1. The oxidation of various phospholipid preparations has been catalyzed by glutathione, cysteine, ascorbic acid, thiamin, riboflavin, pyridoxine, and methylene blue.

2. At pH 3.5 catalytic activity was in the following order: ascorbic acid, glutathione, and cysteine. These 3 catalysts were inactive at pH 7.4.

3. Thiamin, riboflavin, pyridoxine, and methylene blue catalyzed the oxidation of phospholipids at pH 7.4 but not at pH 3.5. These catalysts were more effective with phospholipids prepared from egg yolks than from rat livers.

4. The oxidation of the various systems could be inhibited by carcinogenic hydrocarbons, estrogenic hormones, and certain other compounds.

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The Effect of Heptyl Aldehyde-Sodium Bisulfite on Spontaneous Tumors of the Mammary Gland in Mice*

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Data have been presented (17) which indicate that an indirect correlation exists between the growth rate and fate of spontaneous tumors and the amount of heptyl aldehyde-sodium bisulfite compound injected. This finding applied to doses ranging from 2 mgm. to 6 mgm. per diem. It was further stated that experiments dealing with tumor-bearing mice receiving larger doses were under investigation. The present report includes the complete data for the series of animals injected with graded doses from 2 to 13 mgm. per diem.

Materials and Methods

All mice in this experiment were fed a diet of Nurishmix. Only A strain multiparous mice with spontaneous tumors of the mammary gland were used. This selection was made since the female mice of the A strain develop tumors having the highest degree of malignancy of any of the pure strains of mice in our laboratory. The criteria of malignancy used were: (a) the growth rate of the tumors as indicated by the average size measured in the longest linear diameter, (b) the number of multiple primary tumors, (c) the number and size of pulmonary metastases, and (d) the survival time, in days, of the tumor-bearing mouse after the discovery of its tumor. Two hundred and fifty mice were used as experimental animals with heptyl aldehyde-sodium bisulfite; 120 mice of approximately similar age and size of initial tumors were used as controls.

A daily subcutaneous injection of an aqueous solution of heptyl aldehyde-sodium bisulfite[1] with the indicated dose was given following the weighing of the mouse and the examination of its physical appearance. A particular mouse received either its full dose or none at all. No attempt was made to vary the daily dose with the body weight of the mouse. Each injection in every series consisted of 0.2 cc. in volume. Solutions were made up daily so that 0.2 cc. would contain the desired daily dose for the particular series. In the higher doses employed severe local ulceration occurred and this interfered with subsequent treatment. The highest daily dose used (13 mgm.) is approximately one-half the minimal lethal dose for mice under the laboratory conditions of the present investigation.

The tumors in all mice were measured by vernier calipers in the two greatest diameters at weekly intervals. For the computation of growth rate in this study only the measurement of longest linear diameter was used. This procedure was followed since a too accurate calculation of the "volume" of a tumor is not warranted for one or more of several reasons. These are: (a) the variable thickness of skin over a tumor cannot be accurately determined; (b) the relative amount of stroma and cancer tissue from time to time is variable; (c) the relative amount of necrosis and hemorrhage is also variable, although both processes usually increase with the size of the tumor, but not always; and (d) the presence of liquefaction and subsequent cystic areas interferes with the estimate of amount of the actual living cancer tissue.

Results

With the divided daily dose, many mice received a total of more than 1,000 mgm. of heptyl aldehyde-sodium bisulfite during their survival period. The maximal total dose that any mouse received was 1,614 mgm. (in the 6 mgm. daily series). This mouse (No. 131998) was injected with 6 mgm. 269 times and survived 305 days following the discovery of her tumor. The longest survival time of a mouse after the discovery of its tumor was 439 days, at which time the mouse (No. 141302) was 710 days old. She had received 775 mgm. in a total of 129 injec-

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[1] The heptyl aldehyde-sodium bisulfite was prepared by the National Drug Company from supplies provided by the author. The addition product contained approximately 51 per cent heptyl aldehyde and 49 per cent sodium bisulfite. All doses stated in this paper are in terms of the addition product.

tions. At death there was no evidence of the original tumor or any other tumor Two of the mice, Nos. 143447 and 144262, are still living, free of any mammary tumor at 309 and 218 days, following the regression of their spontaneous tumors.

The data obtained in the completed series of doses of heptyl aldehyde-sodium bisulfite ranging from 2 mgm. to 13 mgm. per diem, are given in Table I and Fig. 1. The degree of deviation from a measure of average or control malignancy obtained in this graded series of doses with the same drug is given in Fig. 1. This measure of malignancy was a composite estimate based upon the following criteria, (a) growth rate of the tumors, (b) survival time of the tumor-bearing mouse, (c) number and size of lung metastases, (d) shape of tumors as an indication of monocentric or polycentric growth, (e) number of primary tumors, and (f) gross and microscopic appearance of tumor, especially the presence or absence of liquefaction.

It may be noted by an examination of Fig. 1 that there is a varying degree of inhibition of spontaneous tumors in all series of mice treated with doses between 2 and 11 mgm. per diem. In mice of the 12 mgm. per diem series a mild degree of stimulation was obtained as indicated, especially in the polycentric growth of tumors. Many of the tumors in this series showed no evidence of liquefaction and the tumors became mulberry in shape.

TABLE 1: SUMMARY OF DATA

Heptyl aldehyde-sodium bisulfite, in mgm.	Number of mice	Average age in days at start	Survival time, in days	Survival time in tumor mice	Age in days at death	Age in days at death, tumor mice	Number with metastases	Per cent with metastases	Number with multiple tumors	Per cent with multiple tumors	Number with regressions	Per cent with regressions	Tumors drained	Per cent of tumors drained
2	5	435.2	69.6	69.6	504.8	504.8	0	0	2	40.0	0	0	1	20.0
3	5	414.8	72.4	72.4	487.2	487.2	0	0	2	40.0	0	0	2	40.0
4	15	390.6	66.6	66.6	457.5	457.5	1	7.1	4	26.7	0	0	7	46.7
Total—2, 3, 4 mgm. series	25	413.5	69.5	69.5	483.2	483.2	1	4.0	8	32.0	0	0	10	40.0
5	25	448.6	55.3	55.3	503.6	503.6	4	16.0	11	44.0	0	0	17	68.0
6	25	413.5	83.9	44.0	493.4	482.8	9	36.0	5	20.0	5	20.0	14	56.0
7	25	419.6	65.9	61.5	487.4	486.9	1	4.0	7	28.0	2	8.0	13	52.0
Total—5, 6, 7 mgm. series	75	427.2	68.4	53.6	494.8	491.1	14	18.6	23	30.6	7	9.3	44	58.6
8	25	422.8	73.5	72.0	498.2	509.7	8	32.0	5	20.0	2	8.0	12	48.0
9	25	456.1	79.7	71.8	537.2	529.2	3	13.1	8	34.6	2	8.0	8	32.0
10	25	404.8	83.9	68.0	488.7	481.9	10	40.0	2	4.7	4	16.0	9	36.0
Total—8, 9, 10 mgm. series	75	427.9	79.0	70.6	508.0	506.9	21	28.0	15	20.0	8	10.6	29	38.6
11	25	393.3	82.8	65.5	477.8	463.2	10	43.4	7	30.4	2	8.0	4	16.0
12	25	450.6	76.5	68.8	527.1	512.4	9	37.5	7	30.4	2	8.0	3	12.0
13	25	433.8	62.9	62.9	496.7	496.7	6	24.0	6	24.0	0	0.0	6	24.0
Total—11, 12, 13 mgm. series	75	425.9	74.1	65.7	500.5	490.8	25	33.4	20	26.7	4	5.3	13	17.3
Total	250	425.2	71.6	64.9	495.9	492.9	61	25.1	66	26.4	19	7.4	96	38.4
Controls	120	349.1	59.2	59.2	409.0	409.0	58	48.2	34	28.3	0	0.0	0	0.0

This table presents data on (I) number of mice in each series, (II) average age of the mice at the time their spontanous tumors were discovered, the start of treatment, (III) survival time—the average number of days the mice lived after the discovery of their tumors, (IV) survival time of mice still showing some cancerous tissue at death, (V) the average age of death for both groups—some showing tumors and some free of tumor at death, (VI) average age at death of those mice showing some cancerous tissue at death, (VII) number of mice showing pulmonary metastases, (VIII) percentage of mice showing pulmonary metastases, (IX) number of mice showing multiple tumors during the extent of the experiment, (X) percentage of mice showing multiple tumors, (XI) the number of mice showing regression of tumors under treatment, (XII) percentage of mice showing regression of tumors, (XIII) number of mice showing cystic areas capable of being drained by mild suction and (XIV) percentage of mice showing "liquefied" tumors. Composite data for (a) 2, 3, and 4 mgm. per diem, (b) 5, 6, and 7 mgm. per diem, (c) 8, 9, and 10 mgm. per diem, and (d) 11, 12, and 13 mgm. per diem are also given.

Discussion

A series of papers (12-17) dealing with the effects of heptyl aldehyde and its derivatives has been published. The essential feature of this effect on spontaneous tumors of the mammary gland in mice is one of liquefaction. This process of liquefaction has been so extensive at times that drainage by sterile puncture and mild suction has been possible.

The present investigation indicates that the extent of liquefaction of spontaneous tumors is determined by the level at which the daily administration of subcutaneous injections of an aqueous solution of heptyl aldehyde-sodium bisulfite has been kept. The maximum degree of liquefaction, 58.6 per cent in the tumors of all treated mice, was obtained in the composite series of 5, 6, and 7 mgm. per diem. The maximum number of instances of complete regression of tumors under treatment (10.6 per cent) was obtained in the composite series of 8, 9, and 10 mgm. per diem. The lowest percentage of pulmonary

metastases (4.0 per cent) was obtained in the composite series of 2, 3, and 4 mgm. per diem. In the subsequent composite series there was a progressive increase in the number of pulmonary metastases, 4 per cent in the 2, 3, 4 mgm. series; 18.6 per cent in the 5, 6, and 7 mgm. series; 28 per cent in the 8, 9, and 10 mgm. series; and 33.4 per cent in the 11, 12, and 13 mgm. series. In all cases, however, the percentage of pulmonary metastases was lower than it was in the controls (48.2 per cent). The number of mice showing multiple tumors did not seem to

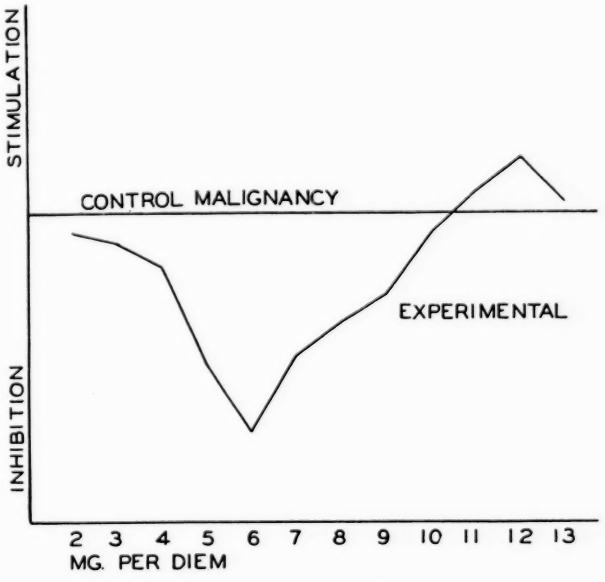

FIG. 1.—This chart presents graphically data on the comparative composite effect of heptyl aldehyde-sodium bisulfite upon spontaneous tumors of the mammary gland in mice. Data for the 120 control mice are given on the straight solid line parallel with the base line, data for the experimental animals (total 250 mice) are given on the curved solid line. The mice are divided according to the amount of the drug received per diem. Deviations from the observations for the control mice can thus be readily seen.

be affected by the experimental procedure of the subcutaneous injection of an aqueous solution of heptyl aldehyde-sodium bisulfite.

Garai (8) has recently reported similar liquefactive processes in spontaneous tumors of mice following the injection of the same drug. He points out that this liquid contains viable tumor cells. We have found that the injection of this liquid into mice belonging to the same strain that gave rise to the spontaneous mammary carcinoma results in a transplantable mammary carcinoma (strain-limited response only).

Garai concludes, however, that the destructive action upon the kidneys and liver exerted by repeated intraperitoneal injections of heptyl aldehyde-sodium bisulfite far outweighs its effect upon the growth and fate of spontaneous tumors. Our findings are not in complete agreement with this conclusion. We have observed the following: (a) a considerable number of control mice with spontaneous tumors show kidney damage; (b) many normal mice without tumors at the age at which spontaneous tumors arise show a high degree of renal disturbance, and (c) the too rapid breakdown of any tissue in mice by x-ray or by any other means is almost always accompanied by renal damage. Since the process of liquefaction in spontaneous tumors is one apparently of breakdown of tissue, it is not unreasonable to conclude that some renal damage should be ascribed to this process rather than exclusively to the heptyl aldehyde-sodium bisulfite itself. Furthermore, we have observed that normal mice without spontaneous tumors can be kept alive in apparently normal health while receiving daily injections of the drug in amounts that will bring about liquefactive processes in neoplastic tissue. These injections did not have any obvious damaging effect on surrounding or internal normal tissues. In view of these conflicting opinions, further work upon the toxic effect of this drug in normal mice free of spontaneous tumors should be done before the final evaluation of the data can be made.

The complicating features of the use of heptyl aldehyde-sodium bisulfite on tumor-bearing mice are: (a) the occasional production of local skin ulcerations with doses of more than 8 mgm. per diem, (b) mild, transitory, unusual irritability, unprovoked squealing and jumping with the larger doses, (c) many mice die of a secondary hemorrhage from the surface of the "liquefying" tumor, (d) secondary infections of liquefying tumors are of frequent occurrence, and (e) liver and renal damage aggravated, perhaps by the too rapid breakdown of neoplastic tissue. Many of these disadvantages may eventually be removed by further research.

It is certain that some effect has been produced on spontaneous tumors of mice by the injection of material at a site remote from the tumor. The observation should warrant further investigation. Further discussion on the use of heptyl aldehyde and its derivatives on experimental cancer by many investigators (1-17) will be forthcoming in a review article.

SUMMARY AND CONCLUSIONS

1. Spontaneous carcinoma of the mammary gland in mice may be influenced by the injection of an aqueous solution of heptyl aldehyde-sodium bisulfite at sites remote from the tumor.

2. This effect may be altered by the amount of the drug administered per diem.

3. There is a graded sequence of inhibition of spontaneous tumors as measured by the criteria employed in this investigation between 2 mgm. per diem and 6 mgm. per diem.

4. There is a reverse sequence of inhibition on tumors between 6 mgm. per diem and 11 mgm. per diem.

5. A daily dose of 12 mgm. per diem actually mildly stimulates spontaneous tumors, especially in polycentric growth.

6. The maximal degree of inhibition of tumors was obtained with 6 mgm. per diem.

7. The maximum number of instances of complete regression of tumors under treatment was 10.6 per cent. This occurred in composite series of 5, 6, and 7 mgm. per diem.

8. The number and size of pulmonary metastases is indirectly proportional to the amount of the drug administered per diem; the minimal number (4.0 per cent of total treated mice) was obtained with 2, 3, and 4 mgm. per diem; the maximal number (33.4 per cent of total treated mice) was obtained with 11, 12, and 13 mgm. per diem.

9. The maximal degree of liquefaction, 58.6 per cent, was obtained with 5, 6, and 7 mgm. per diem.

10. With doses of 10 mgm. and more per diem liquefaction occurred infrequently.

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Further Investigation of Induced Tumors in Fowls

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Transplantations of tumors induced in mammals by chemical carcinogenic agents have shown that these tumors vary in no essential particular from the transplanted spontaneous tumors in the same species. In the hands of the majority of investigators the induced tumors of fowls show an important variation from the spontaneous ones in that they seem to lack a transmitting agent separable from the tumor cell. In order to obtain further material for investigation, a large number of fowls has been given injections of a chemical carcinogenic agent and the resultant reaction studied.

In 1925, Murphy and Landsteiner (12) reported the induction of two typical sarcomas following the repeated injection of a high boiling point fraction of coal tar. The times of appearance were 12 weeks and 20 weeks respectively following the first injection. Single instances of induced tumors in fowls have been reported by Leitch (6), Choldin (3), Mellanby (11), and by Maisin and Dupuis (8). A more extensive study was published in 1933 by Peacock (15) in which he reports that 13 fowls out of 31 injected with coal tar developed sarcoma with an average induction time of 22.5 weeks. A similar number of fowls injected with dibenzanthracene in lard resulted in sarcoma in 10 with an average induction time of 36 weeks. In the same year McIntosh (9) reported a group of experiments in which 13 fowls of the 21 injected with coal tar developed tumors. With Selbie, he (10) later recorded the results of a second series. Benzpyrene in lard used by Rothbard and Herman (17) induced tumors in 3 out of 6 chickens. One of these tumors was accompanied by myelogenous leukemia.

Methods of Induction

In the first series, 21 fowls were given injections of lard containing 0.4 per cent of 1,2,5,6-dibenzanthracene into each pectoral muscle. The initial dose varied between 0.15 cc. and 1.2 cc. Three months later, second injections of 1 cc. of the lard suspension were made into the same areas and subsequently at 2-month intervals each animal received 2 more injections of 0.7 cc. into each breast. The total amount of dibenzanthracene given in the 4 injections over 8 months averages 12.8 mgm. in each location. The chickens in this group were Plymouth Rocks and Rhode Island Reds, between 1 and 2 years of age.

Five out of the 21 chickens developed progressive tumors (23.8 per cent) appearing from 18 to 56 weeks after the first injection with an average time of induction of 36.4 weeks. All of the tumors grew progressively and all metastasized. The results are itemized in Table I.

The length of time required and the small percentage of tumors made this an unsatisfactory result when the objective had been to obtain a large number of tumors from which a number of strains could be started. The following method was next investigated.

The fowls in this series were given a single injection in each breast of 24 mgm. of dibenzanthracene in benzol (0.8 cc. of a 3 per cent suspension). As the same method was used for the several groups making up this series and there was no variation in the results, they will all be grouped together. The fowls used in these and all subsequent tests were Plymouth Rock hens of approximately a year in age. Of the 53 animals injected 48 (90.6 per cent) developed progressive tumors with an average induction time of 5½ weeks. Autografts were made from 23 of these tumors as soon as growth was noted and 22 of the grafts gave progressive tumors.

It is of interest to note that the crystals of the injected dibenzanthracene were found generally in the center of the tumors in amounts suggesting that almost the total amount had remained local. In fact, from one tumor we were able to recover over 70 per cent of the amount injected. There were no metastases in the 12 fowls with tumors which were killed or died before the 7th week following the start of active growth of the tumors but the number with metastases increased from that time till those living over 13 weeks gave a 66.6 per cent rate (Table II).

The difference between time of origin and numbers of tumors produced with the two methods tested is striking. With dibenzanthracene in lard given in 4 doses, the time of origin averaged 36.4 weeks and only 23.8 per cent of the fowls developed tumors. With a single injection of dibenzanthracene in benzol the time of origin of the tumor averaged 5½ weeks and 90.6 per cent of the fowls developed tumors. However, these latter fowls received 24 mgm. in the single injection, while those of the first series averaged only 12.8 mgm. distributed in 4 injections. In contrast with the fact that most of the dibenzanthracene injected with benzol remained local that injected with lard showed only minute scattery traces in the region of the tumors. There was also evidence of extensive

liver damage in a number of the fowls of the latter group while those of the former showed no such reaction. In order to test the possibility that tumor production had a definite relationship to the amount of the inciting agent remaining at the site of injection, the following test was made.

Experiments.—Three groups were included, one in which the dibenzanthracene was given in benzol, one with lard, and a third group was injected with dibenzanthracene in chicken fat where a quick dissemination and absorption would be expected. Each fowl received 0.8 cc. of a 3 per cent dibenzanthracene suspension in each breast which amounted to 24 mgm. in each location or a total of 48 mgm. for each fowl. The results are given in Table III.

Even with comparable doses the induced tumors are more frequent and occur at an earlier period in the fowl injected with benzol suspension than in those given the lard suspension. When the chicken fat was used one tumor was produced and this appeared only after 9 weeks. At autopsy there was little evidence of the dibenzanthracene when it had been given in chicken fat, the lard-injected fowls showed scattered remnants of the material, while the benzol-injected fowls showed a solid clump of the dibenzanthracene crystals at the site of injection.

TABLE I: EFFECT OF SMALL REPEATED INJECTIONS OF DIBENZANTHRACENE IN LARD IN FOWLS

Chicken No.	Total amount * in each breast, mgm.	Result	Time of appearance after 1st injection, in weeks	Metastases	Period of observation in weeks
463	10.2	.....	..	.....	65
471	10.2	.....	..	.....	43
472	10.2	.....	..	.....	65
473	10.48	.....	..	.....	65
465	11.2	.....	..	.....	65
475	11.2	Tumor	27	Heart, lungs, liver, ovary, mesentery	48
477	11.2	.....	..	.....	65
470	12.4	.....	..	.....	38
479	12.4	.....	..	.....	69
480	12.4	.....	..	.....	62
481	12.4	.....	..	.....	59
482	12.4	.....	..	.....	64
456	13.2	Tumor	39	Heart	39
478	13.6	Tumor	56	Liver, ovary	61
468	14.4	.....	..	.....	61
476	14.4	Tumor	18	Liver, ovary, spleen	31
483	14.4	.....	..	.....	56
484	14.4	.....	..	.....	45
485	14.4	.....	..	.....	36
486	14.4	.....	..	.....	57
474	18.6	Tumor	42	Lung, liver, mesentery	67
Average	12.8	5 Tumors 23.8%	36.4	5 100%	55.3

* The amount of the initial injection was varied; the second was 4 mgm. in each breast; the third and fourth were 2.8 mgm. in each breast. These injections were given at about 2½-month intervals.

TABLE II: METASTASES IN FOWLS WITH INDUCED TUMORS

Duration of tumor growth in weeks	Numbers of fowls	Number with metastases	Distribution of metastases						
			Heart	Lungs	Liver	Ovary	Kidney	Spleen	Pancreas
1-3	5	0	..	..	..	..	..	..	..
3-5	6	0	..	..	..	..	..	..	..
5-7	1	0	..	..	..	..	..	..	..
7-9	9	1 (11.1%)	1	1	1	1	..	..	..
9-11	9	4 (44.4%)	1	3	2	2	1	..	..
11-13	12	7 (59.1%)	2	5	5	..	..	1	1
13-15	6	4 (66.6%)	2	3	..	..	..	..	..
Total	48	16 (33.3%)	6	12	8	3	1	1	1

The results when dibenzanthracene in benzol was used, where 90.6 per cent of the injected fowls developed progressive tumors in an average time of 5½ weeks, represented a marked improvement over other methods. In Table IV, these results are compared with those reported by Peacock (15), McIntosh (9), and by McIntosh and Selbie (10). It is difficult to estimate the dosage used in these latter tests but our small series in which comparable amounts of the chemical were given in different solvents indicates that the dosage was not the important factor in determining the result. Rather it would seem that this depended on the amount of dibenzanthracene which remains at the site of inoculation.

Table III: Comparison of the Tumor Producing Property of Dibenzanthracene in Three Solvents

Solvent	Dibenzanthracene, total amount injected *	No. chickens injected	No. positive	Per cent positive	Average time of appearance of tumors, in weeks	Average size of tumors at 8 weeks, in cm.
Benzol	48 mgm.	9	8	87.5	4	4.3 x 3.5
Lard	48 mgm.	5	3	60.0	5	1.5 x 1.2
Chicken fat	48 mgm.	10	1	10.0	9	..

* 0.8 cc. of a 3 per cent solution of dibenzanthracene (24 mgm.) was injected into the pectoral muscles of each breast.

Table IV: Results Compared with Those of Other Studies

Source of data	Material injected	No. chickens	No. tumors	Percentage tumors	Time in weeks
Present series	Dibenzanthracene in benzol	53	48	90.6	5.5
Peacock (15), 1st series	Tar	31	13	41.9	22.5
Peacock (16), 2nd series	Dibenzanthracene in lard	31	10	32.3	36
McIntosh (9), 1st series	Tar in lard	21	13	61.9	?
McIntosh (10), 2nd series	Tar in lard	19	6	31.6	28

Systemic and Distal Effects from Dibenzanthracene Injections

Evidence of systemic effects from the surface application of carcinogenic tar was noted early in the study of these agents (7, 13). The occurrence of tumors at a distance from the site of application of a carcinogenic agent was first noted by Murphy and Sturm (14). Claude (4) has reported extensive liver damage in rabbits following subcutaneous injections of dibenzanthracene in lard and similar systemic effects following applications of tar or pure carcinogenic hydrocarbons have been recorded by other observers (2).

It is difficult to get details from McIntosh's (9) publications on induced tumors in fowls but he states "proliferative changes in sites other than those injected were not an uncommon feature in the experiments." He also observed a "tendency for the leucosarcomata of leukaemias to occur along with the sarcomas" and in a few birds "plain leukaemia was present." It is of interest that McIntosh injected tar mixed with lard. Peacock (15), using in one group an emulsion formed by shaking tar with a soap solution and using whole tar alone in another group, did not note any systemic or distal effects. However, in a third experiment, where fowls were injected in one breast with dibenzanthracene in lard and in the other with lard alone, there were 3 instances of tumor developing in the lard-injected area. There are some observations in our experiments which seem to indicate that the systemic and distal tumor induction is dependent on the amount of dissemination of the carcinogenic agent. This in turn depends on the solvent used. The results were as follows:

Dibenzanthracene in benzol.—There were 53 chickens in the groups described above which received a single injection in each breast of 24 mgm. of dibenzanthracene suspended in benzol and 48 of these developed progressive tumors. Metastases occurred not uncommonly but the autopsies showed no evidence of systemic changes not attributable to this condition. There were 3 instances of tumors in the ovary and, as these occurred in fowls with widespread metastases, no sections were taken. However, in the gross these ovarian masses had the same appearance as the other metastases and did not resemble the primary ovarian tumors observed in the following series.

Effect of dibenzanthracene in lard.—In this group there were 21 fowls which received 4 injections of small amounts of dibenzanthracene in lard at about 2-month intervals and 5 received a single dose in each breast of a larger amount of the agent. The following fowls were found to have lesions other than local reactions to the agent.

Chicken No. 456. There were 4 injections of dibenzanthracene in lard in this animal with a total dosage of 26.4 mgm. and an 8-month period between the first injection and time of death. A gristly tumor was found at the site of injection. The liver and spleen were greatly enlarged and small hemorrhagic areas were noted in the bone marrow, ovary, and kidney. Microscopic examination of the liver showed extensive necrosis and a marked

infiltration of myeloid cells in active division. Diagnosis—myelogenous leukemia.

Chicken No. 478. This fowl had 4 injections with a total dose of 27.2 mgm. of dibenzanthracene. It was killed 14 months after the first injection. There were tumors in both breast muscles. The liver was greatly enlarged with white areas and small hemorrhages on the surface. The spleen was enlarged and the ovary was replaced by a tumor mass. Many nodules from pinhead to pea size were scattered throughout the abdomen. Microscopically the breast tumors were made up of large lymphoid cells. The liver was extensively infiltrated with the same type of cell and the spleen almost uniformly made of them. Similar infiltration was found in the kidney and bone marrow with the blood vessels filled with lymphocytes. The tumor in the region of the ovary and the scattered nodules in the mesentery were composed of dense fibrous tissue with scattered islands of epithelial cells (Fig. 1). Diagnosis—leukemia and fibrous adenocarcinoma of uncertain origin.

Chicken No. 479. The total amount of dibenzanthracene given in 4 injections was 24.8 mgm. and the period of observation was 69 weeks. No local tumors developed. At autopsy the liver was enlarged and the ovary contained a large mass the size of a golf ball. Microscopically this tumor, like that found in chicken No. 478, was composed of dense fibrous tissue with scattered islands of epithelial elements (Fig. 2). Diagnosis—fibro-adenoma of the ovary.

Chicken No. 483. The total dibenzanthracene given in 4 injections was 28.8 mgm. in lard and the time from the first injection till the death of the fowl was 57 weeks. Both the spleen and liver were greatly enlarged, and hemorrhages were scattered through the latter organ. No local tumor developed. The liver was small and cirrhotic. The ovary was replaced by a large tumor mass with innumerable small tumors scattered through the mesentery. The tumors, microscopically, were made up of masses or sheets of cells with little stroma but surrounded by dense fibrous tissue (Figs. 3 and 4). Diagnosis—carcinoma.

Chicken No. 486. The total dibenzanthracene given in 4 injections was 28.8 mgm. and the time from the first injection till the death of the fowl was 57 weeks. Both the spleen and liver were greatly enlarged, and hemorrhages were scattered through the latter organ. Microscopic examination showed few intact liver cells, these being largely replaced by connective tissue with an extensive infiltration and nodules of myeloid cells. Bone marrow and spleen showed a similar infiltration. Diagnosis—myeloid leukemia.

Chicken No. 990. This fowl received 24 mgm. with lard in each breast and was under observation 6 weeks. Tumors developed at the site of both injections. The liver was small, markedly congested, and showed scattered areas of necrosis.

Chicken No. 992. The dosage and time of observation were the same as those in the preceding fowl. The liver was enlarged and showed extensive necrosis with active proliferation of the bile ducts.

Chicken No. 993. The same amount of dibenzanthracene was given this fowl as the 2 preceding ones and the period of observations was 9 weeks. One local tumor developed. An enlarged liver showed extensive necrosis.

Dibenzanthracene in chicken fat.—Among the 10 fowls which were given 24 mgm. of dibenzanthracene in chicken fat at a single injection in each breast muscle, the following 6 showed evidence of systemic effects:

Chicken No. 100A. The period of observation was 18 weeks. One local tumor developed which metastasized to liver, lungs, ovary, and kidneys. The liver showed marked fatty degeneration.

Chicken No. 996. The time between the injection of dibenzanthracene and death of the animal was 7 weeks. No local tumors developed. The liver was enlarged, hemorrhagic, and showed extensive necrosis with some evidence of regeneration.

Chicken No. 997. This animal survived only 3 weeks after the injection of dibenzanthracene. There was a large amount of ascitic fluid and a greatly enlarged liver. This showed marked congestion with evidence of extensive damage to the liver cells. Regeneration was well advanced.

Chicken No. 998. Death occurred 7 weeks after injection. A greatly enlarged liver showed extensive destruction and the spleen was hemorrhagic with some necrosis.

Chicken No. 999. After 7 weeks of survival, the liver was cirrhotic with extensive infiltrations of lymphocytes. There were areas where these cells formed nodules in which many mitotic figures were noted. This is probably a lymphatic leukemia.

From the above information, it may be noted that among the 89 fowls injected with dibenzanthracene evidence of systemic or distal effects were noted in 21 (Table V). All of these were from the 36 fowls in which the hydrocarbon had been administered in either lard or chicken fat. Liver damage was most prominent when chicken fat was used as the solvent, this being present in 60 per cent, and there was one instance of lymphatic leukemia in this group. When lard was used 30.8 per cent showed liver damage, 3 fowls had primary tumors at a distance from the site of injection, and 3 showed evidence of leukemia. The absence of any gross systemic effect in the much larger number of fowls injected with dibenzanthracene in benzol is probably due to the fact that little of the agent was absorbed from the site of injection as previously noted. On the other hand no large local deposits were found where lard was used and what was left in the injected area was widely scattered through the muscle. There was even less of the dibenzanthracene left in the local region when chicken fat had been used and this small amount was spread through a large area. From these observations it seems probable that the development of distal tumors and leukemias are the result of the direct action of the absorbed dibenzanthracene.

Seasonal Variation in Induced Tumors.

Seasonal variations in susceptibility to transplanted tumors is definite, particularly with the Brown-Pearce rabbit tumor (1) and to a less extent with fowl and mouse tumors. Peacock (16) noted a seasonal factor which influenced the rate of growth and transmissibility of induced fowl tumors. There seem to have been no

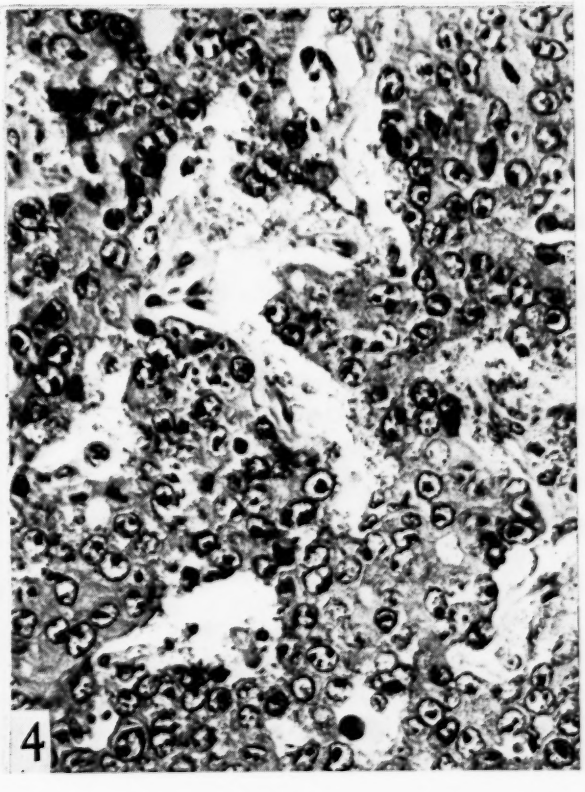

FIG. 1.—Section from a tumor in the region of the ovary. Chicken No. 478. The epithelial elements are surrounded by dense connective tissue. Mag. × 1,000.

FIG. 2.—Section from ovarian tumor in Chicken No. 479. The epithelial elements are more prominent than in the tumor shown in Fig. 1. Mag. × 1,000.

FIG. 3.—Section of a nodule in the peritoneum, with the primary tumor in the ovary. Mag. × 70.

FIG. 4.—A higher magnification of the tumor shown in Fig. 3. Mag. × 500.

definite observations on similar variation in primary induced tumors. However, an analysis of the one large group of fowls in the foregoing tests, where comparable methods were used, suggests a seasonal influence on the induction time.

There were 48 tumors induced by a single injection into each pectoral muscle of 0.8 cc. of a 3 per cent suspension of dibenzanthracene in benzol. Of these chickens, 9 were injected in October, 9 the latter part of December, 22 in January, and 8 between the latter part of February and the first of March. These fowls were from the same source, of the same breed, and of about the same age, and were kept under identical housing and feeding conditions. The average time between the injection and the appearance of a definite tumor for the animals injected in October was 9 weeks, with the range from 6 to 12. The average for the December group was 5.6 weeks with the range between 4.5 and 8 weeks; for the fowls injected in January, the interval was 4.7 weeks ranging from 3 to 6 weeks. The figure for the groups injected in February and March was 4.06 weeks with a low of 3 and a high of 5 weeks. The results are shown graphically in Fig. 5.

Table V: Evidence of Systemic or Distal Effects in Fowls Injected with Dibenzanthracene

Solvent used with dibenzanthracene	Number of chickens	Local tumors	Distal tumors	Liver damage	Leucosis
Benzol	53	48	0	0	0
Lard	26	8	3 (11.5%)	8 (30.8%)	3 (11.5%)
Chicken fat	10	1	0	6 (60%)	1 (10%)
Total	89	57	3	14	4

Taking the extremes of the groups, the October one had only a single instance of a tumor arising as early as 6 weeks while the February-March animals all showed tumors by the 5th week. This lag in the development of tumors in the Fall has been observed in other fowls but as they are of another breed they are not included. Although the numbers are not large for the individual groups, the difference of over a month in induction time between groups is probably significant.

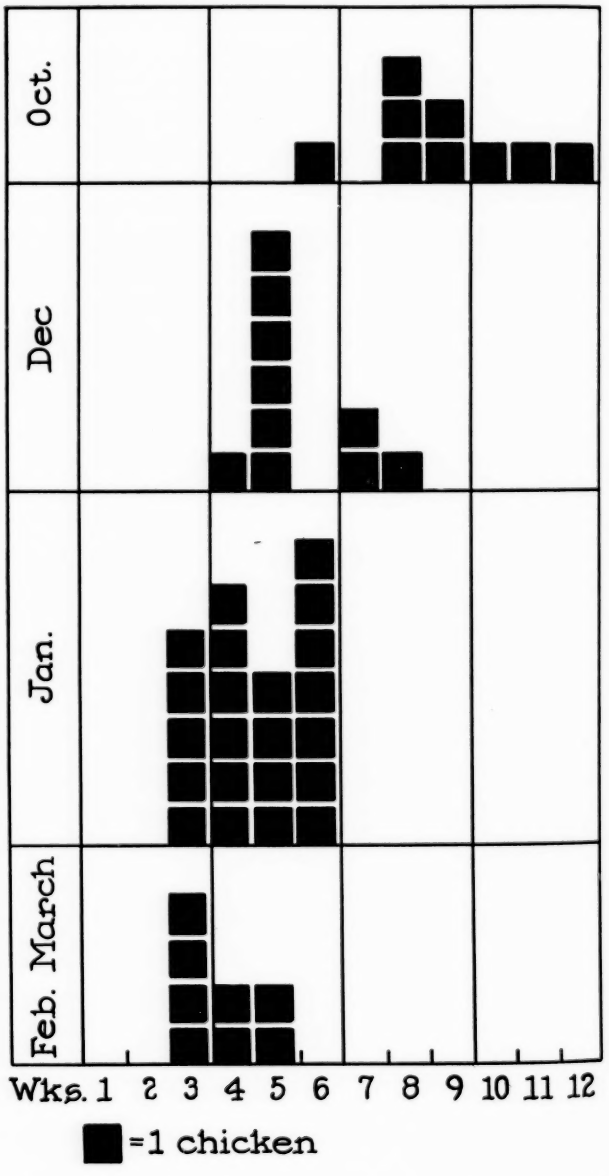

Fig. 5.—The time of appearance of tumors after a single injection of dibenzanthracene in benzol showing a variation in induction time at different seasons.

Discussion

The occurrence of tumors at a distance from the site of injection or applications of a chemical carcinogenic agent has been noted by a number of observers. The first examples were the lung tumors developing in animals following application of tar to the skin (14). Claude [1] found uterine tumors in 2 out of 4 rabbits given subcutaneous injections of dibenzanthracene with doses sufficient to produce extensive liver damage. He also observed atrophy of the testicle and hypertrophy of the breast in male rabbits treated in the same manner. It is a question here as to whether these changes are the result of direct action of the carcinogenic agent or are secondary to the liver damage. There is some evidence that with cirrhosis of the liver there is an interference with the destruction

[1] An unpublished observation referred to by Burrows and Boyland (2).

or inactivation of estrogen with a resultant accumulation which may account for gynecomastia with atrophy of the testicles which sometimes accompanies this condition (5).

As already noted, McIntosh found leukemia and leucosarcomatous tumors at a distance from the site of injection of coal tar and lard. Peacock injected fowls with lard and dibenzanthracene in one breast and lard alone in the other, and reports that 3 fowls developed tumors on the side where only lard had been given. In our series the distal tumors and leukemias occurred only when the carcinogenic chemical was given in solvents more or less readily absorbed, but no systemic effects were noted when the agent was given in benzol. Under the latter condition little of the dibenzanthracene was disseminated. There is no indication as to whether the ovarian tumors developed as the direct effect of the absorbed dibenzanthracene or whether they were secondarily associated with the liver damage.

It is a question as to whether benzol augments the local action of the hydrocarbon or whether the results are attributable to the fact that most of the dibenzanthracene remains as a concrete mass of crystals when this solvent is used. The suggestion that carcinogenic agents act through a combined local and systemic effect is not supported by our findings. Certainly the most prompt response in the fowl occurs when the agents are least disseminated and when no systemic damage is found.

The evidence for seasonal variations in susceptibility to induced tumors is interesting in that it corresponds closely to the variation in susceptibility to transplanted tumors.

Summary

The intramuscular injection of 1,2,5,6-dibenzanthracene in benzol produces tumors in a higher percentage of chickens in a shorter period than when the agent is given with either lard or chicken fat. The average time for induction with the benzol suspensions was 5½ weeks and tumors developed in 90.6 per cent of the 53 fowls of the series.

Data are given which indicate a seasonal variation in susceptibility to induced tumors.

When the dibenzanthracene is given in either lard or chicken fat and dissemination occurs, systemic and distal effects are noted such as damage to the liver, primary tumors of the ovary, and leukemia. There is no evidence of these conditions when benzol is used as the solvent for the hydrocarbon.

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Studies on the Changes in the Lymphoid Tissue of Mice Treated with Carcinogenic and Noncarcinogenic Hydrocarbons*

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It has been shown that treatment with carcinogenic hydrocarbons produces changes in the lymphoid tissue of mice (3). The lymphocytes were found to be decreased, the reticulum cells increased, and there was a deposition of iron-reacting material in the lymph glands. Similar changes were observed after x-radiation in the rat (2). The accumulation of iron in the lymphoid tissue of mice treated with 1,2,5,6-dibenzanthracene endosuccinate has been demonstrated quantitatively (6). These findings were considered in relation to the destruction of red blood cells. A decrease of hemoglobin in the blood with advancing age has been observed in a cancer-susceptible strain of mice whereas in a cancer-resistant strain a similar phenomenon was not found (5).

The aim of the present investigation was to follow the changes in the lymphoid tissue of mice induced by treatment with polycyclic hydrocarbons. It seemed probable that by the examination of the different lymph glands of the same animal after different periods of treatment a clearer picture might be obtained of the changes which occur. For this purpose mice treated with carcinogenic and noncarcinogenic hydrocarbons were subjected to excision of lymph glands at regular intervals, and to splenectomy.

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Method

The hydrocarbons used were 1,2,5,6-dibenzanthracene, 1,2-benzanthracene, and anthracene, which were given as a 0.05 per cent colloidal solution in 1 per cent watery gelatine (0.5 ml. injected subcutaneously in the neck once weekly), while controls received gelatine solution alone, or no injection. The total amount of hydrocarbon given in 40 weeks was 10 mgm. The various series are shown in Table I.

Lymph glands were removed each week from 2 animals of each group under evipan narcosis (4 to 5 mgm. in 1 per cent solution intraperitoneally). The first lymph gland taken was the left inguinal. Every week, 2 other mice of the same groups were operated on similarly, so that at the end of the 10th week the left inguinal gland had been removed from all 20 mice of each group. In the 11th week the right inguinal gland was removed from the mice operated on in the 1st week, and so on. Between the 21st and 30th week the animals were splenectomized in the same order and after the 30th week the left axillary glands were taken. The technic of the operation is simple except for occasional bleeding from the axillary vessels when the adjacent glands are excised. The specimens were fixed in 4 per cent formol-saline, the sections stained with hematoxylin-eosin, and the Prussian blue reaction used for the demonstration of iron. The fol-

Table I: Summary of Injection Series

		Injection	Mice: Number	Age	Sex	Breed
Group	A	1,2,5,6-Dibenzanthracene	20	6-8 weeks	♂	Stock albino
	B	1,2,5,6-Dibenzanthracene	20	2-4 months	♂ and ♀	Stock
	C	1,2-Benzanthracene	20	6-8 weeks	♂	Stock
	D	Anthracene	20	2-4 months	♂ and ♀	Stock
	E	Anthracene	20	6-8 weeks	♂	Stock
	F	Gelatine	20	6-8 weeks	♂	Stock
	G	None	20	6-8 weeks	♂	Stock
	H	None (splenectomy only)	20	6-8 weeks	♂	Stock
			160			

lowing tables and calculations refer to the operative specimens only; the post-mortem findings are treated separately.

Changes in Lymph Glands Removed by Operation

Table II summarizes the changes in the excised lymph glands as regards iron reaction, reticulum cells, lymphoid tissue, and lymph sinuses; the changes, or lack of change, recorded in this table indicate the result of a comparison of the inguinal glands excised on the later occasion with the symmetrical glands examined 10 weeks earlier. The animals treated with

Changes in the Spleens Removed by Operation

The histological examination of the spleens removed by operation showed no marked differences in iron content. The lymphoid tissue in the spleens of the animals treated with the carcinogenic hydrocarbon seemed to be reduced, as in the lymph glands, and this reduction ran parallel to the diminution of weight of the spleen although this weight is greatly influenced by the blood content. Table III shows the splenic weight as percentage of the body weight at the time of splenectomy. The body weights ranged from 20.5 gm. (in group F, gelatine) to 38.5 gm. (in groups

Table II: Changes in Excised Lymph Glands

Injection	Number of animals operated upon		Changes in structure of lymph glands: Increased	Decreased	Dilated	Unchanged	Died during first 20 weeks
1,2,5,6-Dibenzanthracene	38	Iron	11 (6 Albino*)	..	..	9 { 5 Albino (Fe nil in 4); 4 Stock (Fe nil in none) }	18
		Reticulum cells	11 (6 Albino)	..	..	9 (3 Albino)	
		Lymphoid cells	..	17 (9 Albino)	..	3	
		Sinuses	..	..	14 (6 Albino)	6	
1,2-Benzanthracene	19	Iron	14	..	..	3 (Fe nil in 1)	2
		Reticulum cells	12	..	..	3	
		Lymphoid cells	..	4	..	11	
		Sinuses	..	..	4	11	
Anthracene	37 †	Iron	13	..	..	8 (Fe nil in 2)	7
		Reticulum cells	11	..	..	9	
		Lymphoid cells	..	6	..	14	
		Sinuses	..	..	7	13	
Gelatine	15	Iron	3	..	..	7 (Fe nil in 6)	5
		Reticulum cells	3	..	..	7	
		Lymphoid cells	..	1	..	9	
		Sinuses	..	..	3	7	
None	17	Iron	3	..	..	10 (Fe nil in all)	4
		Reticulum cells	4	..	..	9	
		Lymphoid cells	..	2	..	11	
		Sinuses	..	..	3	10	

* Three showed increase of Fe before, and 3 after, splenectomy.
† Ten animals killed (worm infection).

all 3 hydrocarbons showed generally an increase of reticulum cells and an accumulation of iron, compared with the gelatine controls and the untreated animals. The two latter series showed no changes during a period of 1 year which could be attributed to increasing age. The increase in reticular elements was parallel to the deposition of iron. Similar effects have previously been demonstrated by Parsons (4) in mice treated with the water-soluble sodium-1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate. In the present experiments the action was not restricted to the carcinogenic hydrocarbon, but appeared similarly in animals treated with 1,2-benzanthracene and anthracene, while the reduction of lymphoid cells was more pronounced in the groups treated with 1,2,5,6-dibenzanthracene.

A and B, 1,2,5,6-dibenzanthracene), and the means of the groups from 26.9 to 31.2 gm. The individual variation of the splenic weights in each group was large, but in animals treated with the carcinogenic hydrocarbon this weight was significantly smaller than that in all other groups. The animals treated with anthracene and 1,2-benzanthracene do not differ significantly from each other nor from the control groups. The distribution of small (below 150 mgm.) and large spleens (over 500 mgm.) is shown in Table IV. Seven mice receiving 1,2,5,6-dibenzanthracene developed tumors, 6 of them after splenectomy; of these, 5 had small spleens, but 2 spleens were of normal size at the date of extirpation (Table V).

TABLE III: STATISTICAL ANALYSIS OF THE RELATIVE SPLENIC WEIGHTS

	1,2,5,6-Dibenzanthracene			1,2-Benzanthracene			Anthracene			Gelatine			Without treatment			Splenectomized only		
	S.W.* mgm.	B.W.† gm.	Per Cent	S.W. mgm.	B.W. gm.	Per Cent	S.W. mgm.	B.W. gm.	Per Cent	S.W. mgm.	B.W. gm.	Per Cent	S.W. mgm.	B.W. gm.	Per Cent	S.W. mgm.	B.W. gm.	Per Cent
T‡	86	36.1	0.24	576	29.5	1.95	235	35.5	0.66	265	31.5	0.84	315	32.5	0.97	365	33.2	1.10
T	324	31.7	1.02	924	34.5	2.68	375	36.0	1.04	400	32.5	1.26	440	25.5	1.72	340	32.2	1.06
	213	33.2	0.64	482	28.2	1.71	281	23.5	1.22	265	28.1	0.94	465	23.5	1.98	940	32.5	2.89
T	348	38.0	0.92	305	32.9	0.92	178	26.5	0.67	285	20.5	1.39	395	28.5	1.38	514	31.0	1.66
T	54	23.5	0.23	221	32.5	0.68	475	31.2	1.52	540	32.5	1.66	515	24.8	2.07	355	28.5	1.25
T	146	25.0	0.58	193	24.9	0.78	150	28.5	0.53	435	26.7	1.63	453	29.5	1.53	305	29.3	1.04
	282	38.5	0.73	496	27.8	1.78	620	28.7	2.16	215	26.5	0.81	343	30.5	1.12	290	33.2	0.87
	225	33.1	0.68	248	35.7	0.70	340	26.4	1.29	246	23.5	1.05	290	27.5	1.05	285	32.2	0.89
	294	27.2	1.08	198	32.8	0.60	480	27.4	1.75	204	20.5	1.00	315	31.5	1.00	300	32.7	0.92
	300	34.5	0.87	425	33.5	1.27	605	25.0	2.42	...	...	...	505	24.0	2.10	345	32.5	1.06
	210	28.1	0.75	1,126	26.9	4.18	462	24.7	1.87	...	...	...	240	26.5	0.91	185	25.7	0.72
	400	29.1	1.38	380	27.2	1.40	220	26.7	0.82	...	...	...	...	...	...	295	30.0	0.98
T	110	28.4	0.39	275	32.5	0.85	821	19.0	4.31	...	...	...	...	...	...	125	30.0	0.42
T	146	29.2	0.50	...	...	...	450	28.0	1.60	...	...	...	...	...	...	345	32.5	1.06
	225	32.0	0.71	...	...	...	425	26.5	1.60	...	...	...	...	...	...	290	29.5	0.98
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	572	35.4	1.61
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	441	33.0	1.34
	...	...	...	...	...	...	...	...	...	...	...	...	..	...	...	352	30.3	1.16
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	265	32.5	0.82
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	240	29.7	0.81
Means	224	31.2	0.72	450	30.7	1.50	408	27.6	1.56	317	26.9	1.18	389	27.7	1.44	357	31.3	1.13

P. = Probability that the result was obtained by chance. A difference in the means yielding a value for $p < 0.01$ was taken as significant.

1,2,5,6-Dibenzanthracene	P. < 0.01	P. << 0.01	P. < 0.01	P. << 0.01	P. < 0.01
1,2-Benzanthracene		0.9-0.8	0.4-0.3	0.7-0.6	0.2-0.1
Anthracene			0.3-0.2	0.7	0.1-0.05
Gelatine				0.2-0.1	0.9-0.8
Without treatment					0.1-0.05
Splenectomized only					

* S.W. = Spleen weight, mgm.
† B.W. = Body weight, gm.
‡ T = Mouse developing tumor at site of injection.

TABLE IV: DISTRIBUTION OF SMALL AND LARGE SPLEENS

Substance injected	Spleens obtained by splenectomy: Total number weighed	Below 150 mgm.	Above 500 mgm.
1,2,5,6-Dibenzanthracene	15	5	..
1,2-Benzanthracene	13	..	3
Anthracene	15	1	3
Gelatine	9	..	1
None	31	1	5

liver cells. The kidneys appeared normal. Iron in the zona reticularis of the adrenal was observed occasionally.

Of the 40 animals of the anthracene group, 10 were killed because of the presence of worms in the liver; the post-mortem findings in the others were similar to those in the 1,2-benzanthracene group. The gelatine-treated and the untreated animals died mainly of

TABLE V: DATA FROM MICE RECEIVING 1,2,5,6-DIBENZANTHRACENE WHICH DEVELOPED TUMORS *

	A–Body weight in gm. on day of splenectomy	B–Splenic weight in mgm.	B per cent of A	Fe in spleen	Fe in inguinal lymph glands obtained by biopsy: Duration of treatment, in weeks	Fe	Duration of treatment, in weeks	Fe	Change in Fe content
Albino	36.1	86	0.24	++	1	Negative	11	Traces	Unchanged
Albino	31.1	324	1.02	+	2	Negative	12	+	Increased
Albino	38.0	348	0.92	++	6	Traces	16	Traces	Unchanged
Albino	23.5	54	0.23	+	7	Negative	17	Traces	Unchanged
Albino †	25.0	146	0.58	+	7	Traces	17	Traces	Unchanged
Stock	28.4	110	0.39	++	7	Negative	17	+	Increased
Stock	29.2	146	0.50	Traces	8	Negative	18	++	Increased

* The animals developed tumors between the 150th and 200th day of treatment.
† Tumor developed before splenectomy.

CHANGES IN ORGANS EXAMINED POST-MORTEM

The histological picture of glands from animals which died or were killed on account of sickness during the treatment was different from that of specimens removed by operation.

The toxicity of 1,2,5,6-dibenzanthracene was fairly high and the lymphoid tissue at autopsy on animals receiving this compound showed great reduction of lymphocytes and large accumulation of iron. The iron content of the various lymph glands was not always alike; the largest deposition was found usually in the mesenteric glands. The weights of the spleens were far below those of the operative specimens, and ranged from 31 to 123 mgm. In these spleens the deposits giving the Prussian blue reaction were abundant, large amounts of brown pigment were present, and the lymphoid and reticular elements were diminished. The liver was generally pale and soft and showed microscopically fatty degeneration and deposition of iron in the Kupffer cells and also in the liver cells. Degeneration of the tubules and Malpighian bodies of the kidney, and iron deposition in the zona reticularis of the adrenal cortex were found. Degeneration of the spermatogenic cells of the testes was not pronounced in every case. In 2 female mice many big corpora lutea were found in the ovaries.

In the 1,2-benzanthracene group there was generally an increase in the reticular elements in lymphoid tissue, and the splenic weights were within the normal limits; the amount of iron deposition in the peripheral glands was of the same order as in the 1,2,5,6-dibenzanthracene group, but usually no iron could be seen in the affections of the lungs, which showed infiltration and hemorrhage; in the lymphoid organs there were generally no definite pathological changes.

DISCUSSION

The changes in the lymphoid tissue in animals treated with carcinogenic hydrocarbons or bearing primary or grafted tumors were first described by Parsons (3) and the need for further observations on the comparative effect of carcinogenic and noncarcinogenic compounds was pointed out by Parsons and Warren (4). In the present experiments the effect of compounds of these two classes, and of similar chemical structure, was investigated, and repeated examination of the lymphoid tissue of the same animal was secured by using the method of biopsy. No significant difference in the increase of reticulum cells, nor in the development of iron-containing deposits in the specimens obtained by successive operations, was observed between the groups treated with carcinogenic and noncarcinogenic hydrocarbons. The decrease of lymphoid cells was greater in the groups treated with 1,2,5,6-dibenzanthracene, and the greatest reduction was observed in the animals showing toxic effects of 1,2,5,6-dibenzanthracene. The animals treated with 1,2-benzanthracene showed an even more constant increase in the iron content of the glands than did those treated with 1,2,5,6-dibenzanthracene (Table II); one must bear in mind that 1,2-benzanthracene cannot be classed as noncarcinogenic without some qualification, since it has produced an epithelioma (1), and its endosuccinate in association with x-radiation can

produce sarcoma (4), but there is no evidence that anthracene is in any way carcinogenic. Of the 7 mice receiving 1,2,5,6-dibenzanthracene which developed sarcoma, 3 showed an increase, and the remaining 4 no increase, of iron in the lymph glands. It is open to further investigation whether the method of staining the iron and the histological appearance gives a true picture of the free iron contents of the glands and whether this can be taken as an index of blood destruction. The present experiments do not indicate that the accumulation of the deposits giving the Prussian blue reaction is correlated with the development of tumors in mice.

Summary

Changes in a lymphoid tissue induced by administration of carcinogenic and noncarcinogenic hydrocarbons were studied in 160 mice. In order to follow the development of the process the method of biopsy was chosen. In a period extending over one year no definite changes were observed through ageing in the untreated animals or those treated with gelatine. The lymphoid tissue of the animals treated with hydrocarbons undergoes definite changes, consisting of increase of the reticulum cells, decrease of lymphoid cells, dilatation of the sinuses, and increase of deposits giving the iron reaction. The decrease of the lymphoid cells was more pronounced in the animals treated with 1,2,5,6-dibenzanthracene; in respect to the increase of iron deposition there was no qualitative difference between the effect of carcinogenic and noncarcinogenic hydrocarbons. The weights of the spleens of mice treated with a carcinogenic hydrocarbon were significantly smaller than those of others untreated or treated with noncarcinogenic hydrocarbons, but the ranges of the various series overlapped widely.

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Experimental Tumors in Lymph Nodes and in Endocrine and Salivary Glands*

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In recent years, numerous neoplasms of the endocrine glands in the human have been described which have produced abnormal amounts of the natural secretions of these glands. This is true of certain tumors of the thyroid, parathyroid, pituitary, testis, ovary, adrenal, and pancreas. A need has long been felt for experimental material of similar nature in small animals to permit further study of the characteristics of these infrequently occurring tumors, and to have a source of material for the study of abnormal endocrine states. During the last 4 years, we have, therefore, attempted to induce the development of tumors in some of these highly differentiated endocrine glands as well as in the salivary glands and lymph nodes of rats and mice.

Methylcholanthrene was first chosen as the agent because it had been shown by Shear (23), Iball (13), and Twort and Twort (26) to be one of the most active carcinogens. To avoid the necrosis and severe reaction caused by this agent in its pure form, less active materials in the form of 5 per cent 1,2,5,6-dibenzanthracene and 10 per cent methylcholanthrene in cholesterol were also used. These did not produce tumors before the animals died of intercurrent diseases. Radon seeds, crystals of thorotrast, of theelin, and of arsenic trioxide have also been tried, all without success in the production of tumors.

The C3H strain of mice has previously been shown by Andervont (1) to be very susceptible to the development of tumors by carcinogenic chemicals. The ancestors of the animals of this strain used in these experiments were obtained from him. A disadvantage of the C3H strain for the present purpose is the high incidence of spontaneous mammary carcinomas in the females. Males were therefore largely used in these experiments. Occasionally, spontaneous mammary tumors were removed surgically to give the carcinogenic agent a longer period to act. The ancestors of our strain A mice were obtained from the Roscoe B. Jackson Memorial Laboratories at Bar Harbor, Maine. Mice of this strain were found also to be very susceptible to carcinogenic chemicals.

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Technic

Mixtures of methylcholanthrene or of 1,2,5,6-dibenzanthracene with cholesterol were made by heating the mixed crystals to the melting point several times and agitating the container while the substance was in a molten state. In earlier series pellets were formed by the technic described by Shear (23) by drawing the molten substance into glass tubes having a small bore, and expelling the material after cooling. The long rods obtained could be cut into any desired length. The pellet was then inserted into a cannula for introduction into the tissues. In later series the pure methylcholanthrene was melted and permitted to harden. Small pellets were then chipped off and, when used, were introduced into the tissues directly without the use of a cannula.

Implantation was performed in the earliest series by the use of either a steel or glass cannula provided with an obturator. A small pellet was introduced into the end of the cannula, a minute stab wound made in the capsule of the exposed gland, and the cannula introduced to the approximate center of the gland. The pellet was then expelled by advancing the obturator. In later series a pellet about 0.5 mm. in diameter was insinuated with a sharp pointed instrument into the substance of the gland through a small stab wound in the capsule under the magnification provided by a Beebe loupe. The latter method was used for the introduction of crystals of arsenic trioxide, theelin, and thorotrast. The fact that the pellets had been placed accurately in the substance of the lymph nodes and glands was proved subsequently by histological sections.

Thyroid.—A small pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol was implanted into the right lobe of the thyroid gland in 10 mice of the C3H strain. All died of intercurrent disease without the development of tumors. Because of the vascularity and the very thin structure of this gland it was most

difficult to be certain that the agent was placed within its substance and, therefore, the observation was not repeated.

We have been unable to find a report in the literature of the production of a tumor of the thyroid gland at the site of a carcinogenic agent. Dobrovolskaïa-Zavadskaïa and Adamova (9) reported a strain of mice in which there seemed to be a susceptibility to thyroid neoplasia after subcutaneous injection of 1,2,5,6-dibenzanthracene in olive oil or lard at some distance from the thyroid gland.

Thymus.—Pellets of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol were introduced into the thymus glands of 12 mice of the C3H strain. All died of intercurrent disease without the development of tumors. A pellet of pure methylcholanthrene was introduced into the thymus of 4 rats only a few days old, and a pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol into the thymus of 8 additional rats of the same age. None developed tumors, although 1 animal lived almost 3 years after introduction of the agent.

Lewis has reported the development of a lymphosarcoma of the thymus in a mouse of strain C3H which had received a small amount of dibenzanthracene in the pleural cavity 115 days previously.

Pituitary.—A pellet of pure methylcholanthrene was introduced between the lobes of the pituitary gland in 6 rats after exposure of the gland by the technic previously described (12). None developed tumors although the last animal did not die until 584 days after implantation. In a second series of 4 rats a pellet of 10 per cent methylcholanthrene in cholesterol was placed between the pituitary lobes. One animal developed widespread tumors with the histological characteristics of a lymphosarcoma 8 months after implantation, but there was no tumor at the site of implantation of the chemical. No tumor developed in the other 3 rats, although the last animal lived 17 months after treatment.

Radon seeds, two at 1.2 mc. and two at 0.2 mc., were placed between the lobes of the pituitary gland in 4 rats, 2 of which were only 18 days old. They all died within 5 months without tumors.

No experimental pituitary tumors have been described at the site of introduction of a carcinogenic agent. Oberling, Guérin, and Guérin (19) reported 3 adenomas of the pituitary in the rat, one composed of chromophobe cells, another of spongiocytes, and a third of castration cells, which appeared after introduction of a pellet of 3,4-benzpyrene under the pia mater in contact with or within the brain tissue at a site apart from the pituitary. Another of their rats which had received a drop of 0.1 per cent solution of 3,4-benzpyrene in oil in the brain developed an epithelial tumor which invaded the anterior lobe of the pituitary. However, in a later report, these investigators suggest that these tumors may have been of independent spontaneous origin as it was found that hypophysial tumors were more common in normal rats than they had previously supposed.

Weil (27), after injections of styryl 430 into the brains of rats, reported that "neoplastic transformation took place in the pars nervosa and glandular portion" of the pituitary gland. Seligman, Shear, and Alexander (22) have succeeded in producing brain tumors in mice with methylcholanthrene, but have reported no pituitary tumors.

Testis.—A pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol was introduced into the right testis of 10 mice of the C3H strain, and a pellet of 50 per cent dibenzanthracene in cholesterol was similarly used in 5 mice of strain A. No tumors developed, the longest survivor having lived 6 months.

Few experimental tumors of the testis have been reported. Teratomas of the testes have been produced in fowls by injections of zinc chloride by Michalowsky (17), Bagg (2), and Falin (11); and malignant seminomas of the testes have been produced by regeneration after partial castration in fowls by Champy and Lavedan (6).

Pancreas.—A pellet of 5 per cent 1,2,5,6-dibenzanthracene in cholesterol was placed in the pancreatic tissue in 10 mice of the C3H strain and a crystal of pure methylcholanthrene in 7 mice of strain A. No tumors resulted before the death of the last animal 6½ months after introduction of the chemical. No experimental carcinomas of the pancreas have been found reported in the literature.

Adrenals.—The adrenal gland adapted itself very well to introduction of a small pellet within its substance in spite of its small size. The left adrenal was used and was exposed through a left flank incision. A minute hole could be made in the capsule of the gland and a pellet introduced with minimal contamination.

The animals and substances used are summarized in Table I. It is interesting to note that without exception the resulting tumors were sarcomas. The glands were examined at various stages after introduction of a crystal of carcinogenic agent, and it was observed that although the pellet was placed at the center of the gland, the small tract of introduction admitted fibroblasts for the repair process, presumably from the pericapsular connective tissue. The pellet then became surrounded by a wall of fibrous tissue from which the fibrosarcomas developed, and the parenchyma of the adrenal was destroyed by invasion by the tumor.

The only experimental adrenal tumor we have found reported is that of Lacassagne (15). A tumor of the testis was produced in a rabbit 6 months after the introduction of 1,2,5,6-dibenzanthracene in lard. After careful study he suggested that the tumor might be derived from remnants of the adrenal cortex which may occur in the rabbit's testis.

Lymph nodes.—The inguinal lymph node of both rats and mice was found suitable for introduction of various substances by the same technic as was used for the adrenals. This could easily be done through

a skin incision about 5 mm. long and the pellet introduced with the aid of a Beebe magnifying loupe. The animals and substances used are tabulated in Table II.

By examining the nodes in the animals dying of intercurrent diseases at various intervals after the introduction of the carcinogenic pellets, it was observed that the same sequence of histological changes occurred here as in the adrenal. A small focus of necrosis appeared about the pellet in the center of the node and gradually became replaced by fibroblasts entering along the tract of introduction. When tumors developed they were usually fibrosarcomas. In two instances the tumors appeared to be hemangio-endotheliomas. No tumors of the lymphocytic series appeared. The observations on the lymph nodes are tabulated in Table II.

An attempt was made to enhance the action of the methylcholanthrene by producing hyperplasia of the node from a sterile inflammation in the hamstring muscles by the injection of 0.1 cc turpentine in gum arabic. No tumors resulted although the longest survivor lived 21 months.

Beck (3) also found that the action of the carcinogenic chemical is not enhanced by inflammation.

Lewis (16) has reported a lymphosarcoma arising from a lymph node at the site of injection of dibenzanthracene in olive oil in the right axilla of a BA strain mouse, after 237 days, and another tumor apparently composed of lymphoblasts arising from a mediastinal lymph node. Morton and Mider (18) and also Brues and Marble (5) have reported that under appropriate conditions the application of carcinogens to the skin of mice may give rise to lymphoblastoma or lymphatic leukemia in certain strains of mice. A lymphosarcoma occurred in our series of animals following the introduction of 10 per cent methylcholanthrene in cholesterol in the pituitary region.

Salivary gland.—Pure methylcholanthrene in the form of a pellet was introduced into the right submaxillary salivary gland of 4 rats. One epidermoid carcinoma resulted after 196 days. Two of the animals died with abscesses in the salivary glands in the third month after the introduction of the carcinogen. Subsequent experiments have been carried out on mice because of the shorter period usually required for the development of tumors with carcinogenic agents in these animals. A total of 30 mice of strain A has been used. When tumors developed, the animals in many instances were allowed to live until death resulted from the tumor in order to determine whether metastases would occur. Seventeen tumors occurred and, of these, 12 were verified histologically as being epidermoid carcinomas and one a fibrosarcoma. In another 5 animals, death occurred during the night and the ulcerated tumors were eaten off by the cage mates so that the diagnosis could not be confirmed. The earliest tumor developed in 64 days, the latest in 215 days. The average time required for the production of a palpable tumor was 165 days. Ulceration of the tumors occurred early, and death usually resulted in from 2 weeks to 1 month after the appearance of the tumor. Cyst formation with secondary infection and rupture occurred frequently, presumably due to obstruction of the salivary ducts by the tumor. No tumors of the mixed type were found. The tumors all arose in the deeper tissues and were not attached

Table I: Experiments on the Adrenal Gland

Animals	Number	Substance	Tumors	Day of first observation of palpable tumor
C3H mice	5	100% methylcholanthrene	3 fibrosarcomas	160, 166, 178
A mice	9	100% methylcholanthrene	2 fibrosarcomas	263, 280
A mice	17	100% methylcholanthrene + glycerine	None	
A mice	5	Theelin crystals	None	
A mice	10	50% theelin in cholesterol	None	
A mice	10	Thorotrast crystals	None	
Rats	8	100% methylcholanthrene	None	

Table II: Experiments on Lymph Nodes

Animals	No. used	Substance	No. of tumors	Day of first observation of palpable tumor
C3H mice	9	100% methylcholanthrene	5 fibrosarcomas	204, 220, 205, 196, 205
C mice	16	100% methylcholanthrene	8 fibrosarcomas	185 to 328
			2 hemangio-endotheliomas	283, 308
A mice	8	100% methylcholanthrene	5 fibrosarcomas	128, 196, 153, 153, 196
A mice	10	As_2O_3 crystals	None	
A mice	5	50% theelin in cholesterol	None	
A mice	11	Pure theelin crystal	None	
Rats	8	0.1 cc. turpentine in gum arabic in hamstring muscles. 100% methylcholanthrene in lymph node.	None	

to the skin at their first appearance, so that it may safely be assumed that they did not arise from the skin.

At the Third International Cancer Congress in 1939, Steiner (24) reported the induction of epidermoid cysts, squamous cell carcinomas, and carcinosarcomas in the submaxillary gland of rats and mice by the use of 3,4-benzpyrene and methylcholanthrene. Benecke and Schröder (4) have reported the production of polymorphous spindle cell or round cell sarcomas in rats, and sarcomas with 1 squamous cell carcinoma in mice, from the injection of benzpyrene into the parotid glands. Rusch, Baumann, and Maison (21) have also reported squamous cell carcinomas in mice and spindle cell sarcomas in rats following injections of 3,4-benzpyrene and 1,2,5,6-dibenzanthracene dissolved in corn oil into the submaxillary salivary glands.

Because a specific virus has been described as occurring in the submaxillary salivary gland in a large percentage of guinea pigs (7) and also less commonly in rats (25) and white stock mice (14), Dr. Olive Gates has examined a number of normal salivary glands from our stock of mice and rats without finding any intranuclear inclusions which are reported invariably to be found when the virus is present. Injection of a cell-free and bacteria-free filtrate of a submaxillary gland tumor from a mouse produced no intranuclear inclusions in other normal mice of the same strain.[1] This investigation was carried out to determine whether the presence of a salivary gland virus might have enhanced tumor formation in this location.

Discussion

In all the glands used, with the exception of the salivary gland, the parenchymal cells have been either nonsusceptible to the production of tumors, or the connective tissue taking part in the repair process has proved more susceptible to the production of tumors than the more differentiated parenchymal cells. When a tumor of connective tissue origin developed, the death of the animal invariably occurred before a tumor of the parenchymal cells appeared.

We have, as yet, found no solution to this problem in mice or rats, as the glands are so small that even a minute break in the capsule permits fibroblasts of capsular or extracapsular origin to penetrate to the center of the gland along the small channel of insertion and thus to come in contact with the carcinogenic agent. It is possible that the use of somewhat larger animals would eliminate the difficulty occasioned by the premature development of fibrosarcomas, as fibroblasts from the capsule would probably be less likely to penetrate to the center of a larger organ. However, one must be prepared to wait a much longer period of time for the development of tumors from carcinogenic chemicals in the larger animals as judged by the reported periods required for the development of tumors from the skin and subcutaneous tissues in these animals.

The mechanism of the production of malignant tumors by chemical compounds has previously been described by Wolbach (28), and the more recent literature has been reviewed by Cook and Kennaway (8). There is general agreement that foci of necrosis appear which produce a characteristic repair reaction, leukocytic infiltration, and dilatation of capillaries. The reactions we have observed in the tissues are in keeping with these observations and, although the chemical was placed in the parenchyma of the gland, the repair reaction was excited almost exclusively in the connective tissue.

[1] We are indebted to Dr. David Cheever, Jr., for carrying out this part of the experiment in the Department of Bacteriology at the Harvard Medical School.

Summary

Attempts have been made during the past 4 years to produce tumors of the more highly differentiated tissues as represented by the pancreas, lymph nodes, and the thymus, thyroid, adrenal, pituitary, and salivary glands with 1,2,5,6-dibenzanthracene, methylcholanthrene, radon seeds, crystals of thorotrast, of theelin, and of arsenic trioxide in rats and mice. Five fibrosarcomas developed in the adrenal region and 18 fibrosarcomas appeared at the site of implantation of methylcholanthrene in lymph nodes, but all were thought to have originated from the pericapsular connective tissue. Two hemangio-endotheliomas developed from methylcholanthrene implanted in lymph nodes. In only the submaxillary salivary glands were tumors of parenchymatous origin produced. In this location, of 17 tumors produced, 12 epidermoid carcinomas and 1 fibrosarcoma were microscopically verified.

The authors are indebted to Dr. Shields Warren and Dr. Olive Gates for assistance in interpreting the microscopic sections of the tumors.

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Studies on a Transplantable Embryoma of the Mouse*

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A general characteristic of most tumors is their ability to propagate a single cell type to an extent limited chiefly by the survival of the host. In the case of transplanted tumors, it generally appears that the cell type is a homogeneous and self-perpetuating entity which increases in number by division as rapidly as the requisite nutrition can be secured. When tumors show two or more cell types, one type (the stroma cell) may be contributed by the host in response to the growth of the other, malignant type, thus keeping pace in the same way as in hypertrophy of normal tissues. The type of cell may change during the course of time, as when sarcoma arises from stroma cells. Under certain conditions, two tumor cell types may coexist together for a long period (1). When one cell type grows considerably more rapidly, however, it soon outgrows the other (2).

In the case of normal growth from embryonal through adult life a contrary condition exists. Here growth rate is subject to progressively increased retardation, while the growth and development of the various component tissues falls into line according to an intimate regulation. The evidence is accumulating that the normal regulation of development and growth depends upon certain chemical factors.

Malignant tumors of an embryonal or teratoid nature are of great interest since their behavior is like that of other neoplasms, although they are composed of many cell types. The coexistence of numerous cell types suggests that they are subject to some sort of organizing factor which prevents one tissue from outgrowing another.

* Read at the 33rd Annual Meeting, American Association for Cancer Research, Pittsburgh, Penn., April, 1940.

Experimental Observations

Such an embryonal tumor appeared in a female mouse of the C3H strain which had been inbred for three generations in our laboratory, and previously for at least twenty generations.[1] The mouse bearing the tumor had been pregnant at 3 months of age and delivered 3 young which died shortly after birth. Three months later an intra-abdominal tumor was noted; it was subsequently removed and transplanted into other mice of the same strain.

At the time of removal the tumor measured 2.5 cm. in its longest diameter and was heterogeneous in appearance with many small black areas and with no visible necrosis. Microscopically it contained many types of tissue, both embryonal and mature. It appeared to have arisen in the left ovary; the right ovary was normal in appearance.

The tumor was readily transplantable by subcutaneous, intrasplenic, and intrahepatic inoculation of small fragments, and has taken subcutaneously in over 75 per cent of all C3H mice inoculated, both males and females. Transplants have grown to 2 cm. in size in from 6 weeks to 6 months. It has now been transplanted through 13 generations and over 250 tumors have been examined.

In the course of this continued transplantation the neoplasm showed no essential change in type. All individual tumors are composed of some undifferentiated "embryonal" tissue and some mature tissue of diverse types.

The embryonal tissue is more or less readily distinguishable and contains virtually all of the mitotic

[1] By H. B. Andervont of the United States Public Health Service.

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Concentration of mitotic activity in embryonal areas of tumor. Tumor from a mouse treated 9 hours before by subcutaneous injection of 0.03 mgm. colchicine. Mitoses are extremely numerous in the embryonal (darkly stained) areas, and virtually absent elsewhere. Ninth generation transplant from original tumor. Mag. × 80.

Fig. 2.—Islands of embryonal cells with some resemblance to early embryonic epithelium. Second generation transplant. Mag. × 150.

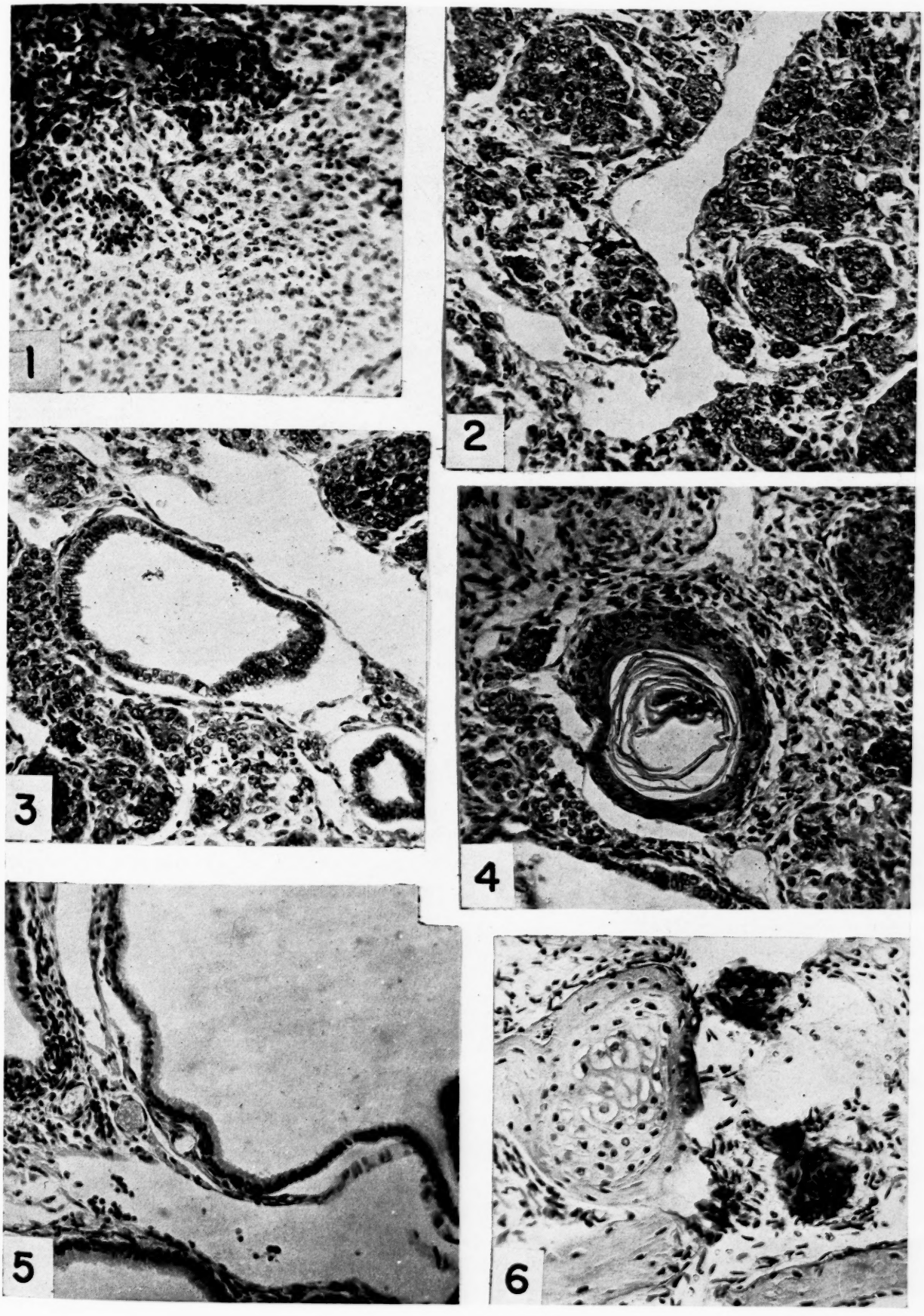
Fig. 3.—Secreting epithelia. Second generation transplant. Mag. × 150.

Fig. 4.—Epithelial pearl formation. Fourth generation transplant. Mag. × 150.

Fig. 5.—Cyst formation. Seventh generation transplant. Mag. × 150.

Fig. 6.—Bone and cartilage. Fifth generation transplant. Mag. × 150.

All sections stained with hematoxylin-eosin.



FIGS. 1 TO 6

activity, which is intense. This is brought out with particular clarity in tumors from colchicine-treated mice (Fig. 1). Most of the embryonal tissue is composed of small cells with little cytoplasm, growing without architectural detail (Fig. 2). Occasionally cells of this type are arranged in structures with some resemblance to neural tubes and these are usually surrounded by mature nerve tissue. Structures resembling trophoblasts, as described in human embryomas by Peyron (3), have not been positively identified.

The mature tissues have included squamous epithelium with pearl formation, pigmented and ciliated epithelia, epithelia containing goblet cells, and various glandular structures, often secreting and sometimes forming cysts containing watery or mucinous material (Figs. 3-5). Smooth muscle, cartilage, bone, and nerve tissue are common. Mitotic activity in all these tissues is very rare, although occasional figures are seen in structures resembling skin or secreting glands. Notably absent are any tissues resembling liver, kidney, or gonads. Throughout all of the mature tissues runs a stroma of more or less differentiated fibroblasts in which mitotic activity is likewise rare. The various tissues showed very little evidence of organization aside from a fairly good stroma-epithelium relationship. Bone has been seen in juxtaposition to connective tissue, and also apparently developing from cartilage (Fig. 6).

Although the general makeup and behavior of the tumor remains constant, great changes in the predominant cell type may occur. In one series of five transplant generations, both nerve and bone have appeared and then disappeared.

In making random selection of tissue for transplantation, an effort was made to secure fragments from remotely separated areas. In some cases obviously differentiated tissues (bone, large heavily pigmented areas, and walls of cysts) have been used. These tissues generally have not taken, or have given rise to extremely slowly growing transplants. Otherwise, random selection of tissue has not resulted in sorting out of tissue types.

Tumors resulting from a series of relatively slowly growing tumors (6 generations in 18 months) show no obvious histological differences from those growing more rapidly (10 generations in the same time). It has been observed in several instances that transplantation of a rapidly growing tumor gives rise to a slowly growing one, from which certain transplants in turn grow rapidly. It is thought that this may be due to varying proportions of embryonal tissue in the fragments used.

A few tissue cultures of the tumor have been made for the special purpose of studying cell division. Good growth of a very pleomorphic type has been obtained with both epithelial and fibroblastic growth. A study of 50 mitoses has failed to show any constant anomaly of division.

In a few preliminary experiments material containing organizers in high concentration [2] was inoculated into our tumors without effecting any apparent change in their architecture.

Metabolic studies by Warburg's method were made on 2 tumors in the series, both of which were composed about equally of embryonal tissue and mature tissue. Most of the mature tissue resembled brain. One of these (No. 209) [3] was an 8th generation tumor; the other (No. 256) [3] was in the 12th transplant generation. The results as shown in Table I and Fig. 7 indicate high aerobic glycolysis and low respiration. It was also observed that succinate had only a slight effect upon oxygen consumption (increasing it about 30 per cent). The respiratory quotients of the 2 tumors were 0.80 and 0.81. Thus the metabolism of this tumor resembles that of most malignant tumors and differs from that usually seen in embryo or brain (4).

TABLE I: METABOLISM OF EMBRYOMA AND OTHER TISSUES * †

Tissue	Oxygen consumption Q_{O_2}	Aerobic glycolysis Q_{O_2}/Q_{CO_2}	Anaerobic glycolysis Q_{N_2}/Q_{CO_2}	Reference
Rat liver	11.5	0.5	4.0	(5)
Rat brain	14	2	14	(6)
Chick embryo, 6 day	11	2	15	(7)
Mouse yolk sac	16	7	23	(8)
Mouse embryoma, transplant No. 209	6.6	11.8	18.5	...
Mouse embryoma, transplant No. 256	7.0	7.0	11.9	...
Mouse sarcoma 180	7.0	14.4	24.7	(9)
Walker rat tumor 256	6.4	11.4	22	(10)

* Numerous additional values for various tissues will be found in the summaries by Burk (4).

† We are indebted for these data to Dr. Francis N. Craig.

DISCUSSION

The occurrence of this ovarian tumor in one mouse following several generations of inbreeding makes it seem probable that the tumor originated as the result of a mutation or a rare embryological accident rather than as a consequence of specific inherited susceptibility. Among 3,000 descendants of one male mouse in our stock, no other ovarian tumors have been seen.

In the light of the fact that relatively uniform tumors often undergo essential changes in the course of transplantation, it might have been supposed that such a complex tumor as this would do likewise.

[2] Obtained through the courtesy of Professor Leigh Hoadley.

[3] Numbers refer to transplanted tumors descended from the original embryoma.

Such a change might take the form of dedifferentiation as the result of cultivation, as mentioned by Lewis (11) in connection with the metamorphosis of myoblastoma to undifferentiated sarcoma; it might occur as the result of the eventual supremacy of a slightly more rapidly growing component; or it might be expected that the random selection of small bits of tissue for implantation would yield a number of different tumor types. The results have shown, however, that this protean tumor reproduces itself with great consistency.

in this tumor seems probable, since the predominant mature cell type shifts in the course of transplantation. This resembles the tendency seen by Bosse (12) for muscle to appear in the metastases from teratoma testis, except that, in the present instance, tendencies of this sort proceed in various directions and are reversible.

The lack of organization in this tumor is especially marked. Falin (13) has seen considerable organization in zinc-induced fowl teratomas in which cartilage and ciliated epithelium created structures strik-

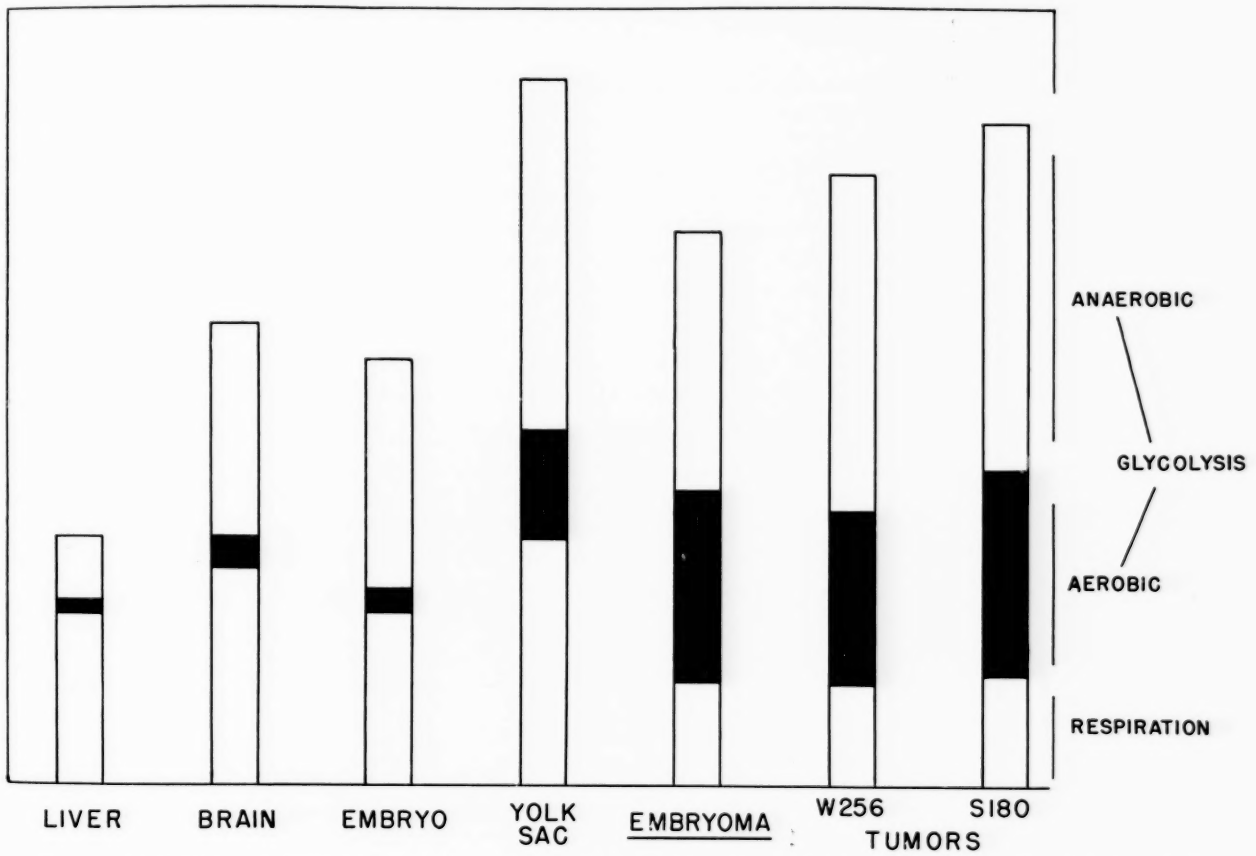


FIG. 7.—Respiratory metabolism of an embryoma transplant (No. 209) and of other tissues. This shows the metabolic similarity of embryoma to other malignant tumors. For data, see Table I.

It seems clear that the most immature cells are responsible for the growth of this tumor, since they contain virtually all of the mitotic activity. In general, rapidly growing tumors show large areas of these immature cells, while in slowly growing tumors they are sparse (Fig. 8). Following growth of this primordial tissue certain cells must mature, and in a few places this maturation is reflected in intermediate stages. The rarity of these intermediate areas suggests that the maturation process is rapid. A few of the matured tissues maintain normal adult mitotic activity, as in the case of skin.

The pluripotential nature of the embryonal cells

ingly like bronchi. Despite the failure of organization, some sort of balancing process obviously keeps cell division and maturation going on at such relative rates that both embryonal and mature tissue have persisted throughout a total period equal to the normal life span of a mouse. In contrast, on the one hand, to the usual situation in transplanted tumors where constancy or dedifferentiation is the rule, and on the other hand, to the maturation of tissues in unison in normal embryogenesis, we see an organization of divided responsibility in which some cells elect to mature while others carry on growth.

This situation might be explained if each cell divi-

sion were uniformly unequal leading to one mature and one immature daughter cell; this would bear an analogy to polar body formation in the ovum. This is an attractive hypothesis, but in the absence of positive evidence from the morphology of mitoses, it would be unwise to carry the analogy too far.

If it were to be supposed that this is an embryo (either parthenogenetically derived or dating back to the previous pregnancy) which has been modified by the loss of some organizing factor, it must be recalled that in the process it has assumed biochemical characteristics (*e.g.*, high glycolysis and low respiratory quotient) common to most true tumors, rather than those of the apparent constituent tissues.

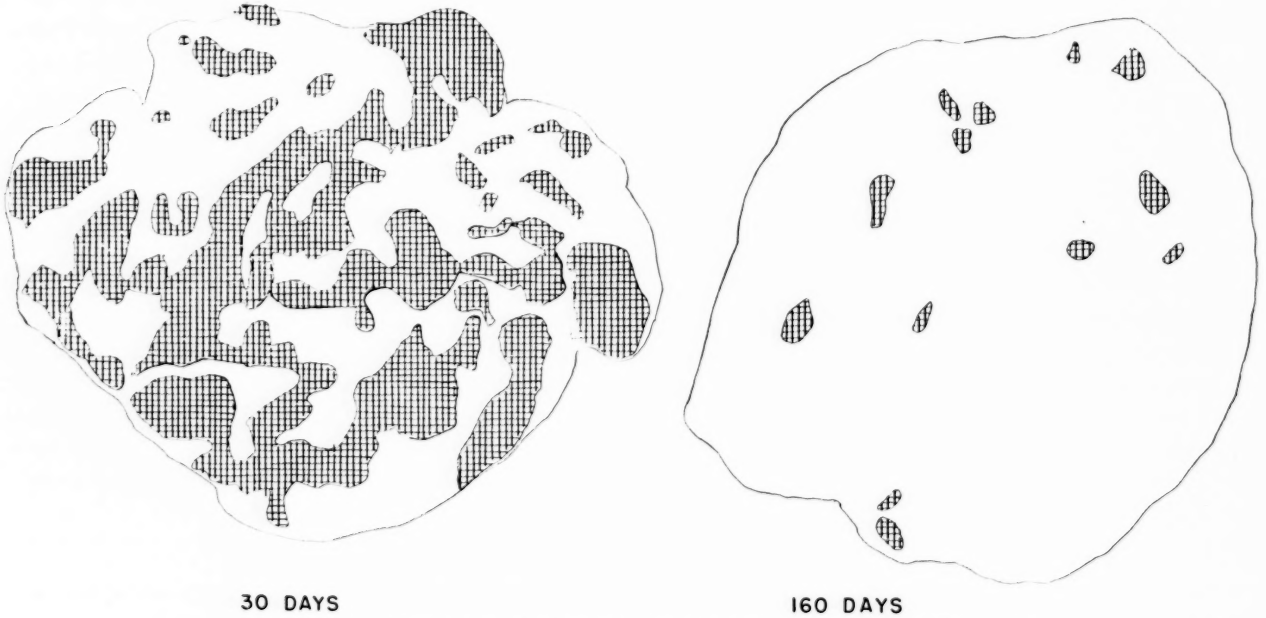


FIG. 8.—Distribution of embryonal tissue in tumors growing at different rates.

This is a map of sections of 2 tumors of about the same size; the one on the left reached this size 30 days after inoculation, and the one on the right grew 160 days after inoculation. The areas of embryonal tissue are shaded.

SUMMARY

An embryoma arising in the ovary of a C3H mouse has been transplanted through 11 serial transplants and maintains its pleomorphic nature *in vivo* and in tissue culture. On biochemical grounds, our studies suggest that it is a true tumor, and that it grows from foci of embryonal cells which divide rapidly throwing off some cells which mature into diverse specialized tissues, and others which retain the ability to grow indefinitely. An analogy is seen between this divided responsibility of daughter cells and polar body formation in ova.

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The Persistence of Growth Inhibition in Young Rats Induced by 1,2,5,6-Dibenzanthracene

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The highly active carcinogenic hydrocarbons have been shown to retard or even inhibit the bodily growth of young rats. The subsequent confirmation by several investigators corresponds so closely to the results of the pioneer work done by Haddow, Scott, and Scott (6) that this observation may be accepted as an established fact.[1]

Perhaps the most remarkable feature of these experiments is the persistence of growth inhibition by the carcinogenic hydrocarbons. The retardation of growth subsequent to a single injection of 10 to 20 mgm. of a highly potent carcinogenic hydrocarbon is described as being extremely prolonged, if not permanent. This is in marked contrast to the action of other growth-retarding agents, such as colchicine and lead, which in one application provoke only a transient interference with growth, followed by resumption of the normal rate of growth.

In view of the earlier prevalent opinion that carcinogenic hydrocarbons are eliminated from the body in a relatively short time following their administration, their prolonged inhibitory effect on growth was surprising. This opinion was based on the studies of Chalmers (4), Chalmers and Peacock (5), Berenblum and Kendal (3), and Hieger (7) which indicated that the period of elimination of administered carcinogenic hydrocarbons was relatively short, the time depending upon the nature of the solvent and the mode of application. In 1937, however, Hieger (8) cautioned against drawing final conclusions, pointing out that the sensitivity of the methods of assay was still under investigation. More recent work, with improved methods, by Peacock and Beck (10) has shown that the period of time required for the elimination of administered carcinogenic hydrocarbons from the body of the experimental animal is significantly longer than formerly assumed.

In view of results of more recent studies of elimination of these compounds the possibility exists that the extremely prolonged growth-inhibitory action of carcinogenic hydrocarbons may be due to the protracted presence of the substances at the site of injection, whence a gradual but uninterrupted resorption takes place. It would be of interest, therefore, to ascertain whether the growth-retarding effect of a compound would continue if the deposit were removed by surgical intervention at a time when unmistakable inhibition of growth had become manifest, or whether the removal of the hydrocarbon would be followed promptly by an increase in the growth rate of the animal. The experiments reported in this paper were made to investigate this problem.

Technical Considerations

The surgical removal of the hydrocarbon several days or weeks after its injection presents serious difficulties. In the experiments of Haddow (6), Lees (9), and others the highly potent carcinogenic compound, emulsified or dissolved in oil or lard, was injected either into the peritoneal cavity or into the subcutaneous tissue of the animal's back or groin. For the purposes of the present study it would have been necessary to remove the oil or lard 15 to 30 days after the injection. It is obviously impossible to remove such material surgically from the peritoneal cavity. Nor would a dissection of the material from the subcutaneous tissue seem feasible because this procedure would introduce a large and indefinite experimental error.

Andervont's (2) experiments on immunization suggested that the injection of the hydrocarbon into the subcutaneous tissue of the rat's tail, with subsequent amputation of the tail cephalad to the site of the deposit, at a suitable time, would be a satisfactory procedure. It seemed to the author that this method would provide a unique opportunity for the study of the resulting changes in the growth rate of the animal.

Much time and work were wasted in a first series

[1] An earlier series of the writer's experiments (1) undertaken for a different purpose, appeared to show that the body growth of young rats had been retarded by exceedingly small doses of carcinogenic hydrocarbons. It is to be admitted freely that this was an erroneous observation due to the omission of certain precautions which at the time did not appear important to the investigator since those studies were conducted with no more than a passing interest in the determination of body growth. In the course of subsequent experiments, undertaken as a check on these results, the author was unable to obtain any distinct reproducible effect. On the contrary, this work confirmed once more the fact established by Haddow (6), by Morelli and Guastalla and by Lees (9) that to induce a striking inhibitory effect a minimum of about 6 to 7 mgm. of a powerful carcinogenic hydrocarbon is needed.—Author.

of 14 experiments on 255 rats with caudal injections of 1,2,5,6-dibenzanthracene in oil. No satisfactory conclusions could be reached because of the high morbidity and mortality of the experimental animals. The chief cause of death was fat embolism resulting, in spite of all possible precautions, in sudden death, or more frequently in chronic infarction of the lung. Infarction of the lung, verified at autopsy, was difficult to diagnose while the animal was alive. This caused embarrassing uncertainties as to whether or not such animals should be included in the calculations.

The prime factor in this condition of embolism and infarction appears to be some peculiarity of the structure of the rat's tail, which favors the direct entrance into the blood stream of fluid media injected into the caudal subcutaneous tissue under pressure greater than that of the venous pressure. In view of the frequency of these complications, the first series of experiments was discarded and the method replaced by one more free from uncertainties and accidents.

Experimental

The following procedure was carried out in 6 experiments with 109 test rats and 58 controls:

A first caudal amputation was performed about 6 to 8 cm. from the tip of the tail. By pushing back the skin a cup 5 to 6 cm. deep was left after dividing and removing the bony portion. This skin cuff was filled to half of its capacity with 10 to 20 mgm. of a thick aqueous paste of crystals of 1,2,5,6-dibenzanthracene. A running suture of two or three stitches closed the cuff. A dressing fixed with mastisol was kept over the wound during the first 24 hours after the operation. In the control rats, operated upon in the same manner and on the same day as the test animals, the skin cuff was half filled with dextrose.

Evidence of the fact that this seemingly more extensive operation is less drastic than caudal injection under pressure is the observation that a decrease in weight, which occurred frequently after injection, was the exception rather than the rule after caudal amputation.

Some uncertainty as to the precise amount of dibenzanthracene applied is, of course, inevitably associated with this method. The knowledge of the exact amount, however, was not considered essential.

After the lapse of a certain time, when impaired growth of the test rats had become unmistakable, a second caudal amputation was performed on both the test and control animals. At this operation a segment 20 to 30 mm. long was removed from the stump of the tail.

Results

Experiment 3, Series II, involving 19 test and 10 control rats, was the first which could be carried to a successful finish. The data, summarized in Table I, are shown graphically in Fig. 1. In spite of the poorly vascularized cicatrix encapsulating the paste of crystals and in spite of the absence of fat which would have aided solution of the compound the results indicated clearly that the dibenzanthracene was absorbed slowly and regularly. The growth of the test animals was definitely retarded.

By the 7th day following the caudal application of 10 mgm. of 1,2,5,6-dibenzanthracene the curve of percentage increase in weight of the test rats was falling steadily below that of the controls. This retardation of growth rate was considerable by the 30th day, when the second caudal amputation was performed to remove the deposit of dibenzanthracene. After the second caudal amputation the direction of the curve of growth rate of the test animals was accelerated. The curve of percentage increase became parallel to that of the controls, but at a lower level, on which it remained until the 110th day, when the experiment was terminated.

Table I: Results of Experiment 3, Series II, Showing Effects of 1,2,5,6-Dibenzanthrene upon Growth of Rats

	Percentage increase of weight		
Days	Control group 10 rats	Test group 19 rats	Difference
1	1st caud. amp., dextrose 20 mgm.	1st caud. amp., dibenzanthracene 10 mgm.	
7	21.3	19.4	1.9
9	29.2	24.2	5.0
14	48.9	39.4	9.5
21	77.5	65.3	12.2
30	107.1	84.5	22.6
	2nd caud. amp.	2nd caud. amp.	
33	110.4	87.5	22.9
42	140.3	116.7	23.6
61	159.5	137.0	22.5
75	190.7	166.3	24.4
91	213.0	190.0	23.0
99	216.0	193.0	23.0
110	230.0	210.0	20.0

Experiments 4, 5, and 6, Series II, involving a total of 90 test rats with 30 controls were carried out to verify this observation.

On account of the highly irritating property of crystals of dibenzanthracene placed in the skin cuff of the rat's tail the slightest slip in asepsis leads to infection and suppuration. This occurred in Experiments 4 and 5. While the stumps of the tails of all the controls healed by primary intention, the majority of the

40 test animals used in these two experiments showed signs of localized inflammation and suppuration.

In Experiments 4 and 5 dibenzanthracene failed to induce the slightest retardation of growth. On this account Experiment 4 was not pursued beyond the 29th day. In Experiment 5, on the 13th day, when the growth rate of the test animals was the same as that of the controls, a second caudal amputation 20 to 30 mm. nearer the body, was performed and 20 mgm. of dibenzanthracene were placed in the new skin cuff. Although the stumps healed aseptically, no inhibition of growth occurred during the next 30 days, at which time the experiment was terminated.

In Experiment 6, Series II, 20 mgm. of 1,2,5,6-dibenzanthracene was placed in the skin cuff after the

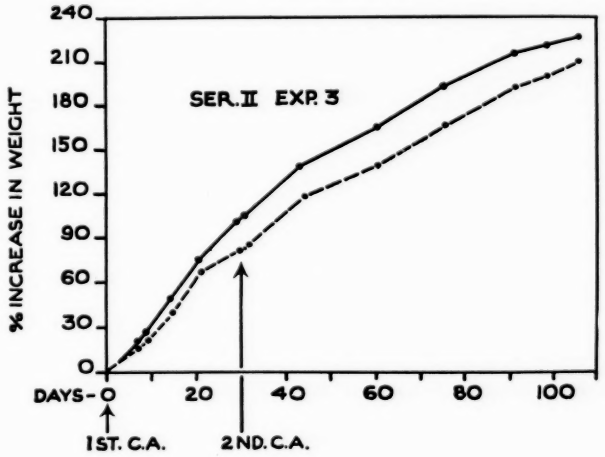

FIG. 1.—Graph of averages of percentage increase in weight of rats used in Experiment 3, Series II. All animals males 40 to 42 days old at start of experiment. Test group, 19 rats, treated with 10 mgm. 1,2,5,6-dibenzanthracene (broken line). Control group, 10 rats, treated with 20 mgm. dextrose (solid line). C.A.-caudal amputation.

first caudal amputation. The results, presented in Table II and Fig. 2, were practically the same as those of Experiment 3. The only noteworthy difference between the two was that in Experiment 6 the retardation of growth rate following the application of dibenzanthracene occurred earlier than it did in Experiment 3. This effect appeared to be attributable to the larger amount of the compound applied in Experiment 6. The stunting of the animals persisted after the removal of the deposit of dibenzanthracene on the 24th day.

DISCUSSION

In the experiments of Series II, described in this communication, there was no significant mortality among the test and control animals, in contrast to the losses which occurred in Series I. In Series II the experiments were started and terminated with a sufficiently large number of animals. The consistent results of Experiments 3 and 6 are therefore regarded as significant evidence that 1,2,5,6-dibenzanthracene applied in the manner described produces retardation of growth of young adult rats and that an effect on

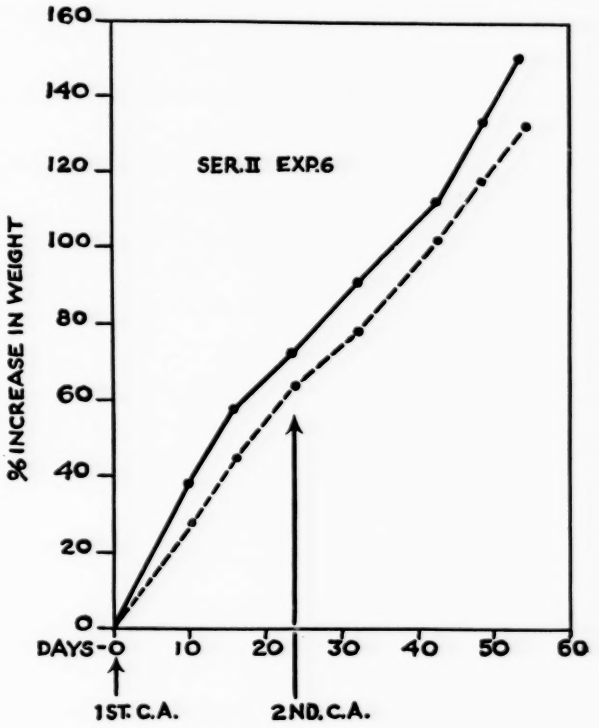

FIG. 2.—Graph of averages of percentage increase in weight of rats used in Experiment 6, Series II. All animals females approximately 40 days old at start of experiment. Test group, 20 rats, treated with 20 mgm. 1,2,5,6-dibenzanthracene (broken line). Control group, 10 rats, treated with 20 mgm. dextrose (solid line). C.A.-caudal amputation.

TABLE II: RESULTS OF EXPERIMENT 6, SERIES II, SHOWING EFFECTS OF 1,2,5,6-DIBENZANTHRACENE UPON GROWTH OF RATS

	Percentage increase of weight		
Days	Control group 10 rats	Test group 20 rats	Difference
1	1st caud. amp., dextrose 20 mgm.	1st caud. amp., dibenzanthracene 20 mgm.	
10	37.5	25.6	11.9
15	54.4	41.1	13.3
24	74.4	58.3	16.1
	2nd caud. amp.	2nd caud. amp.	
31	85.6	70.9	14.7
42	109.7	94.6	15.1
49	134.1	117.9	16.2
54	145.9	130.1	15.8

growth of the animals persists after removal of the compound. Although the results are strictly applicable only to the substance used, namely, 1,2,5,6-dibenzanthracene, they are regarded as evidence confirming the original observation of Haddow (6) concerning

the persistence of inhibition of growth exerted by highly active carcinogenic hydrocarbons.

Two effects were noted in these experiments; (a) permanent dwarfing and (b) progressive retardation of growth rate. These will be discussed briefly.

The dwarfing of rats by dibenzanthracene was irreversible during periods of 204, 180, 142, 110, and 30 days. Apparently this retardation of growth was permanent. On the other hand, the progressive character of growth retardation is probably not an inherent property of the carcinogen. It is the result of a combination of two factors. One factor is the permanent dwarfing effect of the absorbed carcinogen. The second factor, responsible for the progressive course of growth retardation, is the continuous and protracted absorption of the compound from a deposit in the tissues. The moment the deposit is removed by surgical intervention the progressive character of the inhibition of growth ceases.

Although the mode of action of the carcinogenic compounds is obscure, it appears probable that a clearer understanding of their effects may be gained through the knowledge that the general retardation of growth produced by absorbed dibenzanthracene is permanent.

The avoidance of infection is essential for the success of experiments of this type. It seems difficult to escape the deduction from Experiments 4 and 5 that not only the suppuration of the stump but also the induration which follows in the wake of subsiding cellulitis higher in the tail prevented absorption of dibenzanthracene from its deposit in the skin cuff. This deduction is in accord with the opinion of Halsted concerning the genesis of swelling of the arm following operations for cancer of the breast.

Summary and Conclusions

Two series of experiments were performed to investigate the effect of 1,2,5,6-dibenzanthracene upon the growth of young adult white rats.

In Series I, comprising 14 experiments, the hydrocarbon dissolved in oil was injected under pressure into the subcutaneous tissue of the rat's tail. Most of the animals treated in this manner died of fat embolism or of infarction of the lung, as the result of passage of the oily material into the caudal veins. Because of these complications which introduced great uncertainties, this method was discarded.

In Series II, a watery paste of crystals of 1,2,5,6-dibenzanthracene was placed in a skin cuff formed by amputation of a portion of the tail. When inhibition of growth had become unmistakable, 24 to 30 days after the first application of the carcinogen, the deposit was removed by a second caudal amputation. This procedure was entirely satisfactory.

The results of two experiments, in which 59 test animals and 40 controls were used, showed that 1,2,5,6-dibenzanthracene was absorbed gradually and regularly from the skin cuff and produced a persistent inhibition of growth. After removal of the deposit of the compound a rate of growth similar to that of the controls was resumed.

On the basis of these observations, it is concluded that:

1. 1,2,5,6-dibenzanthracene applied locally in the tail of the young adult rat causes a permanent dwarfing of the animal.

2. Progressive inhibition of growth rate is not an inherent property of this carcinogen, but is dependent upon continued absorption of the compound from the local deposit.

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Growth Rates of Nine Inbred Strains of Mice*

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Growth as indicated by weight increase has been studied in young animals of 3 strains of mice with high incidence of mammary carcinoma (A, Dba, C3H) and 6 strains with low tumor incidence (I,[1] JK,[1] C57 black, C57 leaden, CBA, Bagg albino). The mice were kept in a small force-ventilated room at a temperature of not less than 70° F. Their diet was a constant supply of Purina Fox Chow and fresh water, with lettuce and bread and milk twice a week. Females were isolated before the birth of their litters and left for 28 days with their young after birth. Young mice 28 days old were segregated as to sex and put into cages 12 x 7 x 5 inches with not more than 10 mice to a cage. Litters were cut down to 4 at birth, the heaviest and lightest of each sex being retained. Mice were individually marked at birth and weighings made weekly from birth to 10 weeks.

The curves for males of all strains, and those for females of all strains, show that differences exist between strains in rate of growth, but give no indication that these differences are in any way associated with the tumor incidence of the strains. In general, growth rate is lower in strains JK, Dba, and C3H than in the other 6 strains. Differences between strains exist also in the relative rate of growth of males and females of each strain. The curves for the two sexes of one strain follow each other closely for the first 3 or 4 weeks of life and then begin to diverge, the males growing faster than the females. Table I shows the sex differences in grams in each strain at ages between 4 and 9 weeks, and the probability that the difference is due to chance (Fisher's value of p). A probability of 0.01 or less is here taken as indicating a significant sex difference. Probabilities greater than 0.01 are in general not considered as indicating significance because of the variability of the data and the readiness with which weight is influenced by uncontrollable factors of the environment.

TABLE I: WEIGHT DIFFERENCES IN GRAMS BETWEEN MALES AND FEMALES AND THEIR VALUES OF p

Strain	Mammary tumor frequency	Total no. mice		Age in weeks 4	5	6	7	8	9
A	High	64	Difference	...	...	1.2	1.3	1.7	1.9
			p	...	...	0.1	0.1	<0.01	<0.01
Dba	High	100	Difference	...	...	0.3	0.8	2.8	3.5
			p	...	...	0.4-0.3	0.1-0.05	<0.01	<0.01
C3H	High	50	Difference	...	0.3	1.7	3.0	3.1	...
			p	...	0.8-0.7	0.05-0.02	<0.01	<0.01	...
I	Low	133	Difference	0.2	0.7	1.4	2.2	...	...
			p	0.7-0.6	0.1	<0.01	<0.01	...	...
JK	Low	35	Difference	0.1	1.1	1.4	2.7	...	...
			p	0.9-0.8	0.01	0.02	<0.01	...	...
C57 black	Low	91	Difference	1.0	1.7	2.4	...	...	...
			p	0.02-0.01	<0.01	<0.01	...	...	...
C57 leaden	Low	50	Difference	1.2	2.3	4.7	...	...	...
			p	0.05	<0.01	<0.01	...	...	...
CBA	Low	33	Difference	1.0	2.0	2.9	3.7	...	...
			p	0.3	0.02-0.01	<0.01	<0.01	...	...
Bagg albino	Low	48	Difference	0.3	1.1	2.2	2.8	...	...
			p	0.6-0.5	0.02	<0.01	<0.01	...	...

* This investigation was aided by a grant from the Finney-Howell Research Foundation.

[1] Breeding stock supplied through the kindness of Dr. L. C. Strong, Yale University School of Medicine; stock of other strains obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The high tumor strains A and Dba first show a significant sex difference in weight at 8 weeks. The

third susceptible strain, C3H, has a p value of between 0.05 and 0.02 at 6 weeks and of less than 0.01 at 7 weeks. Since the value at 6 weeks represents a sharp drop below that at 5 weeks, it may represent a significant difference. The 6 resistant strains first show a significant sex difference at 5 or 6 weeks. (In C57 black it may be as early as 4 weeks, where p is between 0.02 and 0.01). A given mean difference is more likely to be significant if the samples available are large. Column 3 of Table I shows the sizes of the samples in each strain at the critical ages. In strains CBA and JK the samples are relatively small. In CBA it is probable that a significant difference would appear at 5 weeks (where p is now less than 0.02) if the sample were larger. The 3 susceptible strains, however, have relatively large samples and it is clear that the observed difference between strains in time of appearance of a significant sex difference in weight is not due to the differences in sample sizes. It is a real inter-strain difference which is apparently correlated with susceptibility to mammary gland cancer.

TABLE II: MEAN AGES AT FIRST PARTURITION OF FEMALES OF FIVE STRAINS OF MICE

Strain	Number of females	Mean age in days at birth of first litter
A	37	97.4 ± 4.81
Dba	28	95.7 ± 4.19
I	31	99.8 ± 4.58
JK	16	99.4 ± 3.95
A—from Bittner, (1)	..	91.0
C3H—from Bittner, (2)	..	92.0
Dba—from Murray, (3)	..	104.5

When litter mates of both sexes are put into breeding pens at the age of 4 weeks, the ages of females at the time of the birth of their first litter give an estimate of the age at which puberty occurs in whichever sex matures more slowly. The mean ages at first parturition of females of strains A, Dba, I, and JK are given in Table II, together with data on A, Dba, and C3H from the literature. There is no indication here that A, Dba, and C3H, with a later age of sex divergence in weight, undergo puberty later than strains JK and I, with an earlier age of sex divergence. It is thus improbable that the observed difference in relative growth is to be accounted for by a difference in the time of onset of puberty.

DISCUSSION

It is becoming increasingly clear that malignant growth is quantitatively rather than qualtitatively different from normal growth, and that it may be looked upon as normal growth lacking normal control. The normal growth characteristics of animals with a strong tendency towards the malignant change, such as the susceptible strains of mice studied, are thus of some interest to the question of the origin of malignancy. The correlation which appears in Table I between mammary cancer susceptibility and late age of sex divergence in weight may be interpreted as indicating (if growth in males be taken as showing the normal growth capacity of the strain under prevailing conditions) a higher relative growth rate at 4 or 5 weeks of age in females of the susceptible strains than in females of the resistant strains. A fuller interpretation of the results and their significance to the cancer problem must await experimental work under conditions which alter mammary cancer susceptibility.

SUMMARY

Growth of young mice of 9 inbred strains, kept under similar conditions, was observed by weekly weighings of individuals from birth to 10 weeks of age. Three strains (A, Dba, C3H) having a high incidence of spontaneous mammary gland carcinoma in breeding females, first show a significant difference in weight between the sexes at 8, 8, and 7 weeks of age respectively. In the 6 remaining strains (I, JK, C57 black, C57 leaden, CBA, Bagg albino), all with a low incidence of mammary carcinoma, significant sex difference in weight appears at 5 or 6 weeks of age. These observations are taken to indicate a correlation between mammary cancer susceptibility and the normal growth rate of young animals in inbred lines of mice.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

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7,9,10-Trimethyl-1,2-benzanthracene and 8,9,10-trimethyl-1,2-benzanthracene, prepared from 7-methyl-1,2-benzanthraquinone and 8-methyl-1,2-benzanthraquinone by established methods, will be tested for carcinogenic activity. New methods for the synthesis of the previously known 7-methyl-1,2-benzanthracene and 8-methyl-1,2-benzanthracene are also described.—R. N. J.

BACHMANN, W. E., and J. M. CHEMERDA. [Univ. of Michigan, Ann Arbor, Mich.] THE SYNTHESIS OF 4-METHYLCHOLANTHRENE AND 5-METHYLCHOLANTHRENE. J. Organic Chem., 6:50-53. 1941.

4-Methylcholanthrene and 5-methylcholanthrene (numbering system of *Chemical Abstracts*) have been synthesized by established methods and will be tested for carcinogenic activity. On Fieser's system of ring numbering these compounds are 22-methylcholanthrene and 23-methylcholanthrene respectively.—R. N. J.

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Two independent syntheses of 1-methylchrysene and 3-methylchrysene are described, together with new procedures for obtaining 2-methylchrysene and 4-methylchrysene.—R. N. J.

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A new method for the synthesis of the highly carcinogenic 9,10-dimethyl-1,2-benzanthracene is described. Attempts to synthesize 1,2,9,10-tetramethylanthracene have failed; this compound would be of considerable interest as it is related both to 9,10-dimethyl-1,2-benzanthracene and to 9,10-dimethylanthracene, which latter compound is the simplest carcinogenic hydrocarbon known.—E. L. K.

BERNSTEIN, S., W. J. KAUZMANN, and E. S. WALLIS. [Princeton Univ., Princeton, N. J.] THE RELATIONSHIP BETWEEN OPTICAL ROTATORY POWER AND CONSTITUTION OF THE STEROLS. J. Organic Chem., 6:319-330. 1941.

Relationships between optical rotatory power and chemical structure in the sterol series cannot be established in a manner similar to that which has been used with success in carbohydrate chemistry. The effect of a change in structure on the rotation can be calculated on the basis of certain established structural features of the sterol molecule and the hypothesis that when two centers of asymmetry are spacially well separated (separation greater than 8 A.), they do not appreciably influence each other's contribution to the optical rotation of the molecule. A system of notation is established using cholestane as a reference compound. Extensive tables of observed and calculated optical rotation for many sterol derivatives are included.—R. N. J.

BONSER, G. M., and L. M. WAINMAN. [Univ. of Leeds, England] A FURTHER EXPERIMENTAL STUDY OF THE ANTAGONISM BETWEEN ONE MALIGNANT TUMOUR AND THE APPEARANCE OF ANOTHER IN THE SAME ANIMAL. J. Path. & Bact., 52:263-266. 1941.

A group of 31 pure strain female mice (R III strain, mammary cancer incidence 63% of all females living more than 7 months) were painted weekly with 0.3% methylcholanthrene. A parallel group of 30 similar female mice were kept as controls. Comparison was made of the latent period of the skin tumor and mammary tumors.

The authors conclude that no antagonism exists between the presence of a tumor of one organ and the development of a tumor in another organ. On the contrary, there seems to be evidence of some facilitation. Thus warts appeared earlier in mice which finally developed mammary cancer than in those which did not, and the appearance of mammary cancer was earlier in the series painted with methylcholanthrene than in the untreated animals.—I. H.

BOYLAND, E., A. A. LEVI, E. H. MAWSON, and E. ROE. [The Roy. Cancer Hosp. (Free), London] METABOLISM OF POLYCYCLIC COMPOUNDS. 4. PRODUCTION OF A DIHYDROXY-1:2:5:6-DIBENZANTHRACENE FROM 1:2:5:6-DIBENZANTHRACENE. Biochem. J., 35:184-191. 1941.

Rats and rabbits were maintained in metabolism cages on a diet containing 0.008% of dibenzanthracene. The introduction of the dibenzanthracene and also the same amount of 3,4,5,6-dibenzcarbazole into the diet increased the proportion of both ethereal and neutral sulfur in the urine. The urines were fluorescent and from the urine of rabbits to which dibenzanthracene was fed dihydroxy dibenzanthracene was isolated by adsorption on kieselguhr and by butyl alcohol extraction of the acidified urine. The compound was purified through its diacetyl derivative and other characteristic derivatives were prepared. The absorption spectra of the dihydroxy dibenzanthracene and the corresponding dihydroxy dibenzanthraquinone were compared with dibenzanthraquinone and with 1,5- and 2,6-dihydroxy anthraquinones. The isolated dihydroxy dibenzanthracene was not carcinogenic to mice either when painted or injected so that it would appear that rabbits are able to inactivate 1,2,5,6-dibenzanthracene.—E. B.

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FRANSEEN, C. C., J. C. AUB, and C. L. SIMPSON. [Collis P. Huntington Memorial Hosp., Boston, Mass.] EXPERIMENTAL TUMORS IN LYMPH NODES, AND IN ENDO-

CRINE AND SALIVARY GLANDS. Cancer Research, 1: 489-493. 1941.

The authors have made attempts over a period of 4 years to produce tumors of more highly differentiated tissues as represented by the pancreas, lymph nodes, thymus, thyroid, pituitary, and salivary glands in rats and mice with 1,2,5,6-dibenzanthracene, methylcholanthrene, radon seeds, crystals of thorotrast, of theelin, and of arsenic trioxide. Five fibrosarcomas developed in the adrenal region but were not of adrenal origin and 18 fibrosarcomas appeared at the site of implantation of the methylcholanthrene in the lymph nodes but all were thought to be of pericapsular origin. Two hemangio-endotheliomas developed from methylcholanthrene implanted in lymph nodes in mice. In only the submaxillary salivary glands were tumors of parenchymatous origin produced. In this location, of 17 tumors produced, 12 epidermoid carcinomas and 1 fibrosarcoma were microscopically verified.—Authors' abstract.

HOCH-LIGETI, C. [Roy. Cancer Hosp. (Free), London] STUDIES ON THE CHANGES IN THE LYMPHOID TISSUE OF MICE TREATED WITH CARCINOGENIC AND NON-CARCINOGENIC HYDROCARBONS. Cancer Research, 1: 484-488. 1941.

Changes in the lymphoid tissue induced by administration of carcinogenic and noncarcinogenic hydrocarbons were studied in 160 mice. In order to follow the development of the process the method of biopsy was chosen. In a period extending over one year no definite changes were observed through ageing in the untreated animals or those treated with gelatine. The lymphoid tissue of the animals treated with hydrocarbons undergoes definite changes, consisting of increase of the reticulum cells, decrease of lymphoid cells, dilatation of the sinuses, and increase of deposits giving the iron reaction. The decrease of the lymphoid cells was more pronounced in the animals treated with 1,2,5,6-dibenzanthracene; in respect to the increase of iron deposition there was no qualitative difference between the effect of carcinogenic and noncarcinogenic hydrocarbons. The weights of the spleens of mice treated with a carcinogenic hydrocarbon were significantly smaller than those of others untreated or treated with noncarcinogenic hydrocarbons, but the ranges of the various series overlapped widely.—Author's summary.

MURPHY, J. B., and E. STURM. [Rockefeller Inst. for Med. Research, New York, N. Y.] FURTHER INVESTIGATIONS OF INDUCED TUMORS IN FOWLS. Cancer Research, 1:477-483. 1941.

The intramuscular injection of 1,2,5,6-dibenzenthracene in benzol produces tumors in a higher percentage of chickens in a shorter period than when the agent is given with either lard or chicken fat. The average time for induction with the benzol suspensions was $5\frac{1}{2}$ weeks and tumors developed in 90.6% of the 53 fowls of the series.

Data are given which indicate a seasonal variation in susceptibility to induced tumors.

When the dibenzanthracene is given in either lard or chicken fat and dissemination occurs, systemic and distal effects are noted such as damage to the liver, primary tumors of the ovary, and leukemia. There is no evidence of these conditions when benzol is used as the solvent for the hydrocarbon.—Authors' summary.

NEWMAN, M. S., and J. A. CATHCART. [Ohio State Univ., Columbus, Ohio] THE ORIENTATION OF CHRYSENE. J. Organic Chem., 5:618-622. 1940.

Nitration of chrysene yields only 1 mononitrochrysene which, on reduction and hydrolysis, yields a product identical with the known 6-chrysenol. These reactions support the previously accepted but incompletely established hypothesis that chrysene is attacked most readily at the 6-position. A bibliography of nitro- and amino-chrysene is included.—R. N. J.

RILEY, J. F., and A. B. WALLACE. [Univ. of Edinburgh, Edinburgh] STIMULATION AND INHIBITION OF CONNECTIVE TISSUE IN MICE FOLLOWING INJECTION OF BENZPYRENE. Brit. J. Exper. Path., 22:24-28. 1941.

Groups of mice were given single injections of 0.5 mgm. benzpyrene in 1.0 cc. olive oil. Group A were each injected twice weekly with 0.5 cc. chick embryo extract; group B were injected twice weekly with 0.5 cc. M10 propionaldehyde solution, while group C served as controls. Sarcomas occurred in 42% of group A, 22% of group B, and 36% of the controls. Injection of propionaldehyde alone did not induce sarcomas in mice.—E. B.

WEISS, J. [Durham Univ., King's Coll., Newcastle-upon-Tyne, England] FORMATION OF IONS FROM COMPOUNDS WITH CONJUGATED DOUBLE BONDS: HYDROCARBON SALTS. Nature, London, 147:512. 1941.

The preparation of salts of coronene, 1,2-benzperylene, 3,4-benzpyrene, and anthracene with perchloric, sulfuric, and pyrophosphoric acids, is reported. These salts are formed from the hydrocarbons by an oxidizing agent such as chromium trioxide, potassium ferricyanide, or hydrogen peroxide, in the presence of the fairly concentrated strong acids at room temperature. Anthracene perchlorate, which is formulated as $[C_{14}H_{10}]^{+}\,[ClO_4]^{-}$ occurs as dark brown crystals and its acetone solutions are good conductors of electricity. It is suggested that knowledge of these ions may assist in understanding the biological action of the hydrocarbons.—F. L. W.

Hormones

EISEN, M. J. [Columbia Univ., New York, N. Y.] TUMOR INHIBITION ASSOCIATED WITH SECRETORY CHANGES PRODUCED BY ESTROGEN IN A TRANSPLANTED MAMMARY ADENOCARCINOMA OF THE RAT. Cancer Research, 1:457-464. 1941.

Subcutaneous injections of estrogen in doses of 0.166 to 0.2 mgm. in sesame oil twice weekly in the inbred August strain of rats bearing a regularly growing transplantable adenocarcinoma of the breast inhibited tumor growth. Tumors in treated rats were significantly smaller than in control animals. The growth rate of tumors in treated as in untreated rats is wholly independent of the age or sex of the animals. Estrogenized animals in general showed a loss of body weight. Reduction in weight produced by a caloric restriction of approximately 50% was also associated with a decrease in size of transplanted tumors. The growth rate of 2 rapidly proliferating transplanted spindle cell sarcomas in inbred rat strains was not inhibited by estrogen, despite loss of weight in the treated animals.

Lactational changes in the transplanted mammary cancers were produced in a large majority of female and male rats that survived 21 days after transplantation and received 8 doses of estrogen. This was the minimal quan-

tity of female sex hormone required to produce active secretory changes in the tumors. The lactation phenomena varied in extent in different tumors. Some contained microscopic focal areas of secretion distributed at random, while others were extensively transformed into large cystic spaces containing milk-like fluid. It cannot be excluded that some degree of inhibition of tumor growth resulted from the transformation of potentially proliferating tumor into functioning tissue as a result of adequate hormonal stimulation. In one treated animal a lactating metastasis in the lung was observed.

No alteration was observed in the growth rate of transplanted tumors arising from implants of an estrogenized tumor of diminished rate of proliferation, but without evidence of secretion. The latent period of tumors originating from transplants of grossly lactating tumor tissue was considerably prolonged. In both instances the ensuing tumors reproduced the structure of untreated growths.

Pregnancy and lactation, testosterone propionate, and castration exerted no demonstrable influence on the growth of structure of the mammary adenocarcinoma.—Author's abstract.

LIPSCHÜTZ, A., and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] PREVENTION OF EXPERIMENTAL FIBROIDS BY A CORTICAL HORMONE. Lancet, 1:568-570. 1941.

Tablets of deoxycorticosterone acetate implanted under the skin of castrated female guinea pigs simultaneously with tablets of estradiol prevented the formation of uterine fibroids and reduced extragenital fibroids to a minimum. Extreme development of the uterus and uterine bleeding, which normally follow prolonged estrogen absorption, were inhibited. The cortical hormone prevented the occurrence of purulent peritonitis with uterine localization. The amount of deoxycorticosterone necessary for inhibiting the action of estradiol is about 3 times the amount of the latter. The tumor-preventing action of deoxycorticosterone appears to be greater than that of testosterone or progesterone.—F. L. W.

LOEB, L., and V. SUNTZEFF. [Oscar Johnson Inst., St. Louis, Mo.] THE EFFECTS OF ESTROGEN ON THE MAMMARY GLAND OF MICE INJECTED DURING PREGNANCY, LACTATION, AND RETROGRESSION. Cancer Research, 1: 439-445. 1941.

The changes observed in the mammary gland of mice injected with very large doses of estrogen during late stages of pregnancy or at the beginning of lactation, and continued for various periods not exceeding 3 months, are in principle similar to those observed normally following pregnancy and lactation, except that localized and scattered processes of stimulation in the gland, consisting of hypertrophy, mitotic proliferation, and secretion, are associated with the retrogressive changes, which latter predominated.

There was no significant difference in these effects of estrogen in the medium to high cancer rate strain A and the low cancer rate strain CBA. Carcinomatous change in the mammary glands was not observed under these experimental conditions.—Authors' abstract.

SUNTZEFF, V., M. MOSKOP KIRTZ, H. T. BLUMENTHAL, and L. LOEB. [Oscar Johnson Inst., St. Louis, Mo.] A COMPARISON BETWEEN THE INCIDENCE OF MAMMARY GLAND CARCINOMA AND CANCER AGE IN MICE INJECTED WITH ESTROGEN AND IN NONINJECTED MICE OF DIFFERENT STRAINS. Cancer Research, 1:446-456. 1941.

There exists, in general, a parallelism between the cancer incidence of the mammary gland in breeding control mice of various strains and the readiness with which the cancer rate can be increased in these strains in nonbreeding female mice, or the occurrence of mammary gland carcinoma can be induced in male mice by injections of estrogen. This parallelism is due to the fact that in all of these cases we have to deal with the same mechanism; namely, the production of carcinoma through the action of endogenous or exogenous ovarian hormones acting on a substratum with a definite inherited degree of growth tendency in the mammary gland.

In breeding control mice belonging to strains with the highest incidence of mammary gland carcinoma, the cancer rate was approximately as high in the first as in the later age classes; in strains with a weaker inherited tendency to the development of mammary gland carcinoma, and in nonbreeding mice even of high tumor rate strains, the cancer incidence increased in the direction from the first to the third (oldest) age class. Likewise, the stronger the dose of estrogen injected and the greater the inherited tendency to cancer, the earlier the mammary gland carcinoma appeared in the various strains. This indicates that cancer in general appears earlier in life the greater the intensity with which the causative factors act.—Authors' abstract.

WOLLNER, A. [Columbia Univ., New York, N. Y.] ESTROGEN, TESTOSTERONE AND GROWTH HORMONE IN THE PRODUCTION OF ATYPICAL EPITHELIAL GROWTHS IN THE HUMAN CERVICAL MUCOSA. J. Clin. Endocrinol., 1:228-233. 1941.

Previously Wollner had reported that estrogen given to castrate women induced growth in the columnar epithelium of the cervix, that simultaneous administration of estrone and progesterone produced growth of squamous epithelium of the cervix, that long-continued use of estrogen can result in the clinical and histologic picture of endocervicitis and erosion. Search for a counteractant to "estrone hyperfunction" led to trial of testosterone propionate as treatment for endocervicitis in one woman, in whose case biopsy had shown a typical condition. In 6 weeks a total of 750 mgm. of testosterone propionate was injected in progressively larger doses given twice weekly. Biopsies after the 3rd and 6th weeks showed change to a dense anemic structure with fewer glands and conversion of active tall columnar cells to a nonsecretory low columnar type. Near the external os squamous epithelial elements were observed to change to what strongly resembled a beginning malignancy, the epithelium apparently breaking through the basement membrane in several places. Two ovariectomized women received injections of testosterone propionate and estradiol benzoate for 6 weeks, total doses being, respectively, 104,000 r.u. of estradiol benzoate and 700 mgm. of testosterone propionate; and 96,000 r.u. of estradiol benzoate and 750 mgm. of testosterone propionate. Biopsies were held to show invasion of the stroma and glands by squamous epithelium. Another woman received growth hormones (Parke, Davis & Co., antuitrin growth) plus doses of estrogen and androgen similar to those described above, with even greater incitation of growth.

In 2 other women, combined treatment with estrogen, progesterone, and growth hormone also caused atypical cervical growth, whereas such a state was held not to occur in an undefined number of women given only estrogen and progesterone.

Discussion is made relative to cervical tumors in mice receiving androgens and estrogens. Ten photomicrographs are shown.—J. B. H.

Viruses

DOLJANSKI, L., G. GOLDHABER, M. PIKOVSKI. [Cancer Labs., Hebrew Univ., Jerusalem, Palestine] INACTIVATION OF THE CAUSATIVE AGENT OF FOWL-LEUKOSIS BY X-RAYS. Nature, 147:481. 1941.

The fowl leukosis employed was the hemocytoblastosis strain T of Engelbreth-Holm. Blood was withdrawn from animals at the height of the infection, citrated, and irradiated on a concave slide covered with a mica cover slip, and sealed with paraffin. While the virus was definitely destroyed by a dose of 4,000,000 r, no effect was produced by 2,000,000 r. With 3,000,000 r the results varied (three negatives to two positives). The dosages required to inactivate the agent of fowl leukosis thus lie within the same range as those which have been found to inactivate the few other viruses (fowl plague, infectious papilloma of the rabbit, and vaccinia) of which the resistance to irradiation has been determined.—R. J. L.

MOTTRAM, J. C. [Mount Vernon Hosp., Northwood, Middlesex, England] VIRUS INCLUSIONS, BLASTOGENIC AGENTS AND CILIATES. Nature, 147:479. 1941.

A culture of *Aspidisca* was subjected to a temperature of 40° C. for 1 minute. Six days later many abnormal organisms were observed. When stained by the Feulgen technic they were seen to contain cytoplasmic accumulations of minute particles, suggestive of virus bodies. At the same time they exhibited signs of increased cytoplasmic viscosity. In many cases the micronuclei failed to stain by the Feulgen method, and there occurred unstained bands along the length of the macronuclei. In spite of subculturing, the abnormal ciliates ceased to divide, and all were dead by the 22nd day. Attempts to infect normal organisms, and others subjected to the action of carcinogenic agents, were unsuccessful.—R. J. L.

Genetics

HOWARD, A. [McGill Univ., Montreal, Canada] GROWTH RATES OF NINE INBRED STRAINS OF MICE. Cancer Research, 1:503-504. 1941.

Growth of young mice of 9 inbred strains, kept under similar conditions, was observed by weekly weighings of individuals from birth to 10 weeks of age. Three strains (A, Dba, C3H) having a high incidence of spontaneous mammary gland carcinoma in breeding females, first show a significant difference in weight between the sexes at 8, 8, and 7 weeks of age respectively. In the 6 remaining strains (I, JK, C57 black, C57 leaden, CBA, Bagg albino), all with a low incidence of mammary carcinoma, significant sex difference in weight appears at 5 or 6 weeks of age. These observations are taken to indicate a correlation between mammary cancer susceptibility and the normal growth rate of young animals in inbred lines of mice.—Author's summary.

Radiation

MACKENZIE, K., and H. J. MULLER. [Inst. of Animal Genetics, Univ. of Edinburgh, Edinburgh] MUTATION EFFECTS OF ULTRA-VIOLET LIGHT IN DROSOPHILLA. Proc. Roy. Soc. s. B., 129:491-517. 1940.

The finding that wave lengths of ultraviolet light, which are effective in inducing mutations, begin in the region between 320 and 300 mμ, supports the conception that it is absorption by the nucleic acid which is principally responsible for the mutations induced by ultraviolet radiation. Activation of nucleic acid by ultraviolet light does not result in breakage of chromosomes, but in alteration of the distinctive gene substance. The mutations induced are considered to result from a transference of energy from the nucleic acid to the protein of the gene substance. These mutations are thus distinct in their mode of origin from both the gross and the minute rearrangements of chromosomes, such as are produced by x-rays. The authors support the suggestion of Bauer and others that cancer may originate as the result of somatic cell mutations involving alterations of genes, rather than by imbalance of whole chromosomes as Boveri postulated.—R. J. L.

Biochemistry and Nutrition—Chemotherapy

ALAPY, H. [Roy. Hungarian Palatin-Joseph Univ., Budapest, Hungary] THE PERSISTENCE OF GROWTH INHIBITION IN YOUNG RATS INDUCED By 1,2,5,6-DIBENZANTHRACENE. Cancer Research, 1:499-502. 1941.

Two series of experiments were performed to investigate the effect of 1,2,5,6-dibenzanthracene upon the growth of young adult white rats.

In Series I, comprising 14 experiments, the hydrocarbon dissolved in oil was injected under pressure into the subcutaneous tissue of the rat's tail. Most of the animals treated in this manner died of fat embolism or of infarction of the lung, as the result of passage of the oily material into the caudal veins. Because of these complications which introduced great uncertainties, this method was discarded.

In Series II, a watery paste of crystals of 1,2,5,6-dibenzanthracene was placed in a skin cuff formed by amputation of a portion of the tail. When inhibition of growth had become unmistakable, 24 to 30 days after the first application of the carcinogen, the deposit was removed by a second caudal amputation. This procedure was entirely satisfactory.

The results of two experiments, in which 59 test animals and 40 controls were used, showed that 1,2,5,6-dibenzanthracene was absorbed gradually and regularly from the skin cuff and produced a persistent inhibition of growth. After removal of the deposit of the compound a rate of growth similar to that of the controls was resumed. On the basis of these observations, it is concluded that: 1. 1,2,5,6-dibenzanthracene applied locally in the tail of the young adult rat causes a permanent dwarfing of the animal. 2. Progressive inhibition of growth rate is not an inherent property of this carcinogen, but is dependent upon continued absorption of the compound from the local deposit.—Author's abstract.

BAUMANN, C. A., E. G. FOSTER, and P. S. LAVIK. [Coll. of Agric., Univ. of Wisconsin, Madison, Wis.] THE EFFECT OF CERTAIN CARCINOGENS ON VITAMIN A IN THE LIVER. J. Nutrition, 21:431-444. 1941.

Intraperitoneal injections of colloidal dibenzanthracene increased the rate of depletion of vitamin A from rat livers and also appeared to influence the entrance of vitamin A into the livers. The lack of correlation between carcino-

genicity of a compound and its effect on vitamin A was indicated by the fact that methylcholanthrene, benzpyrene, and 1,2-benzanthracene were less effective than dibenzanthracene in reducing vitamin A storage. Butter yellow and carbon black did not affect hepatic vitamin A. The livers of rats bearing spontaneous mammary adenofibromas contained more vitamin A than the livers of normal control rats. The vitamin A content of livers of rats with tumors induced by methylcholanthrene was greater than that of dibenzanthracene-treated nontumorous animals.—F. L. H.

BOYLAND, E. [The Roy. Cancer Hosp. (Free), London] EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER. Chem. & Indust., 60:219-220. 1941.

Four classes of substances have been found to inhibit tumor growth; *viz.,* 1) citral, heptaldehyde, and their metabolism products, particularly malonic acid; 2) the phosphotungstic acid precipitate of ox heart muscle extract and many natural bases which might occur in such an extract; 3) catalase inhibitors such as nitrosophenols; 4) aromatic diamines, particularly 4,4-diaminodiphenylether. —E. B.

DICKENS, F., and H. WEIL-MALHERBE. [Royal Victoria Infirmary, Newcastle-upon-Tyne] METABOLISM OF NORMAL AND TUMOUR TISSUE. 19. THE METABOLISM OF INTESTINAL MUCOUS MEMBRANE. Biochem. J., 35:7-15. 1941.

The metabolism of different parts of the intestines of rats and mice has been determined by the Warburg technic. The mucous membrane of the duodenum, jejunum, ileum, and colon all have aerobic glycolysis even when the tissue is suspended in serum; the muscularis of the upper part of the rat's jejunum also shows aerobic glycolysis. The R.Q. of rat jejunum mucosa is also low (0.78-0.93). Intestinal tissue like synovial membrane therefore has the type of metabolism which was at one time considered to be specific for tumor tissue.—E. B.

FRASER, R. W., A. P. FORBES, F. ALBRIGHT, H. SULKOWITCH, and E. C. REIFENSTEIN, JR. [Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, Mass.] COLORIMETRIC ASSAY OF 17-KETOSTEROIDS IN URINE. J. Clin. Endocrinol., 1:234-256. 1941.

In normal individuals and in a wide variety of endocrine disorders colorimetric assays were made according to the method of Callow for 17-ketosteroid compounds. The method allowed detection of 88% of known amounts of such steroids which were added to urine. The extensive data and relative simplicity of the method in comparison with bioassay serve to establish tentative standards as follows for levels of urinary excretions in the endocrine states:

	mgm.		mgm.
a) Normal male	14.0	e) Panhypopituitarism (either sex)	0.0
b) Normal female	9.0	f) Gonadectomized male	9.0
c) Female with Addison's disease	0.0	g) Gonadectomized female	9.0
d) Male with Addison's disease	5.0		

Values were also obtained for pregnancy, acromegaly, Cushing's disease, adrenal tumors, precocious puberty, arrhenoblastoma, dysgerminoma, hypothyroidism, and various nonendocrine states such as malnutrition, gastrointestinal disease, anemia, and infections. The authors hypothesize that all urinary 17-ketosteroids are derived from the adrenal cortex and testes, 9 mgm. and 5 mgm. respectively, as the daily excretion due to adrenals and testes. They believe that Cushing's disease is due to hyperadrenocorticism. Assays were also made after administration of chorionic gonadotropin and desoxycorticosterone acetate.—J. B. H.

GUSBERG, S. B., P. ZAMECNIK, and J. C. AUB. [Collis P. Huntington Memorial Hosp., Boston, Mass.] THE DISTRIBUTION OF INJECTED ORGANIC DISELENIDES IN TISSUES OF TUMOR-BEARING ANIMALS. J. Pharmacol. & Exper. Therap., 71:239-245. 1941.

Diselenide diacetic acid and diselenide n-dibutyric acid were administered daily for 15 days by intravenous injection to strain A (Bar Harbor) mice which had been inoculated 2 weeks previously with sarcoma 180. The greatest concentration of selenium was found in the lungs with lesser amounts in kidney, spleen, and liver. After administration of similar selenium compounds containing mercury, more selenium was found in the tissues, the greatest concentration being in the kidneys, then in the liver and lungs. Although addition of mercury to the selenium compounds appeared to alter the excretion of selenium thus causing greater retention in the tissues, histological evidence of extensive kidney damage was not obtained. Selenium injected intravenously as diselenide aliphatic compounds into strain A mice (groups of 6, 5, and 5 animals) or fed in "toxic wheat" to strain C57 mice (8 animals) failed to inhibit the growth of sarcoma 180 (29 control animals) or to cause concentration of selenium in the tumors.—F. L. H.

RUSCH, H. P., and B. E. KLINE. [Univ. of Wisconsin, Madison, Wis.] THE INHIBITION OF PHOSPHOLIPID OXIDATION BY CARCINOGENIC AND RELATED COMPOUNDS. Cancer Research, 1:465-472. 1941.

The oxidation of phospholipids prepared from egg yolk or rat liver was catalyzed by the addition of glutathione, cysteine, ascorbic acid, thiamin, riboflavin, pyridoxine, and methylene blue. The experiments were performed in an ordinary Warburg manometric apparatus and the rate of oxidation followed over a 4-hour period. In general, 3 mgm. of phospholipid, 0.5 mgm. of catalyst, and 50 to 200 μgm. of "inhibitor" incorporated into 2 cc. of the appropriate buffer were the levels employed. At pH 3.5 catalytic activity was in the following order: ascorbic acid, glutathione, and cysteine. These catalysts were inactive at pH 7.4. Thiamin, riboflavin, pyridoxine, and methylene blue catalyzed the oxidation of phospholipids at pH 7.4 but not at pH 3.5. These latter were more effective with phospholipids prepared from egg yolks than from rat livers. The oxygen uptake of 3 mgm. lipid prepared from rat liver in the presence of 0.5 mgm. ascorbic acid varied from 150 to 200 cu. mm. over a 4-hour period. The oxidation of the various systems was inhibited by all the carcinogenic compounds tested. The degree of inhibition decreased from 94% to 30% in the following order: p-dimethylaminoazobenzene, aminoazotoluene, benzpyrene, methylcholanthrene, dihydroestrone, 9,10-dimethyl-1,2-benzanthracene, estrone, and dibenzanthracene. Various noncarcinogenic compounds also inhibited this system but, in general, the degree of inhibition was considerably lower

than that obtained with the carcinogens. The exceptions to this rule were noted with carotene and dihydroxydiphenyl where 81% and 82% depression of the oxygen uptake was observed. Since the carcinogens are soluble in fats and are water-insoluble, these data appear to substantiate the theory that the mechanism of carcinogenesis could be explained as an interference with lipid metabolism. A disturbance in the system described might be one step in a series responsible for cancer formation.—Authors' abstract.

STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] THE EFFECT OF HEPTYL ALDEHYDE-SODIUM BISULFITE ON SPONTANEOUS TUMORS OF THE MAMMARY GLAND IN MICE. Cancer Research, 1:473-476. 1941.

The results of daily injections of graded doses of heptyl aldehyde-sodium bisulfite in aqueous solution into tumor-bearing mice of the Strong A strain have led to the following conclusions: 1. Spontaneous carcinoma of the mammary gland in mice may be influenced by the injection of an aqueous solution of heptyl aldehyde-sodium bisulfite at sites remote from the tumor. 2. This effect may be altered by the amount of the drug administered per diem. 3. There is a graded sequence of inhibition of spontaneous tumors as measured by the criteria employed in this investigation between 2 mgm. per diem and 6 mgm. per diem. 4. There is a reverse sequence of inhibition on tumors between 6 mgm. per diem and 11 mgm. per diem. 5. A daily dose of 12 mgm. per diem actually mildly stimulates spontaneous tumors, especially in polycentric growth. 6. Thus the maximal degree of inhibition of tumors is obtained with 6 mgm. per diem. 7. The number and severity of pulmonary metastases is indirectly proportional to the amount of the drug administered per diem; the minimal number (4.0% of total treated mice) was obtained with 2, 3, and 4 mgm. per diem; the maximal number (33.4% of total treated mice) was obtained with 11, 12, and 13 mgm. per diem. 8. The maximal degree of liquefaction, 58.6%, was obtained with 5, 6, and 7 mgm. per diem. 9. With doses of 10 mgm. and greater per diem liquefaction occurred infrequently.—Author's abstract.

Comparative Oncology

JACKSON, E. B., and A. M. BRUES. [Collis P. Hungtington Memorial Hosp., Boston, Mass.] STUDIES ON A TRANSPLANTABLE EMBRYOMA OF THE MOUSE. Cancer Research, 1:494-498. 1941.

An embryoma arising in the ovary of a C3H mouse has been carried through 13 serial transplants without losing its pleomorphic character. All tumors contain undifferentiated "embryonal" tissue with high mitotic activity and mature tissues of various types which appear to arise from the embryonal tissue and do not grow to any extent. It appears that the growing embryonal tissue throws off some cells which mature into specialized tissues and others which retain the ability to grow; an analogy is seen between this divided responsibility of daughter cells and polar body formation in ova. The embryoma has the metabolic activity of a malignant tumor. —Authors' abstract.

Clinical and Pathological Reports

Etiology

DAVIS, H. H. [Omaha, Neb.] THE POSSIBLE ETIOLOGIC RELATIONSHIP BETWEEN A SINGLE TRAUMA AND MALIGNANT TUMOR. Am. J. Surg., 51:641-652. 1941.

Trauma reveals many malignant tumors, but whether it can actually cause a malignant tumor is an unsettled point. In the discussion following the paper the statement was made that in over 175,000 cases of accident claims made to a railroad there had never been a claim for an injury producing or aggravating a malignancy.—H. G. W.

GOECKERMAN, W. H., and L. F. X. WILHELM. [Los Angeles, Calif.] ARSENIC AS THE CAUSE OF CANCER OF MUCOUS MEMBRANE. Arch. Dermat. & Syph., 42: 641-648. 1940.

A man who took Fowler's solution from 1904 to 1914 developed palmar and plantar keratoses in 1918. In 1935 he developed a papilloma of the ureter and papillomatous lesions of the bladder, which were removed, and disclosed crystals of arsenic. The probability of arsenic crystals remaining in the tissues for 21 years is not discussed.—H. G. W.

MUIR, R. [Univ. and Western Infirmary, Glasgow, Scotland] THE EVOLUTION OF CARCINOMA OF THE MAMMA. J. Path. & Bact., 52:155-172. 1941.

Although the proportion of cases of mammary carcinoma in which the evolution of malignancy can be followed is comparatively small, a considerable number of such cases was studied and the following conclusions reached. The origin of malignancy is frequently multicentric and there may be independent foci in ducts or in acini or in both. Also, the process is often a gradual one and may be seen at different stages in the same breast. "It represents a gradually accelerating hyperplastic growth attended by de-differentiation—the growth, histioid at first, becomes purely cellular." Furthermore, the malignant process often affects a given area in a diffuse manner, the change being regional rather than focal. The diffuse origin of cancer raised the question as to whether there occurs a transference, in an autocatalytic fashion, of growth-stimulating substance from malignant to nonmalignant cells. No clear evidence was found that such a transference occurs, although the question is left entirely open. The facts are interpreted as showing that the carcinoma represents the end result of a disturbance produced by one causal agent. No evidence was found of a dual causation; *e.g.*, of a diffusely acting hormonal agent leading to hyperplasia followed by another agent, such as a virus, causing focal malignancy.—A. H.

PELLER, S., C. S. STEPHENSON, and C. G. SOUDER. [Johns Hopkins Univ., Baltimore, Md.] CANCER IN ITS RELATIONS TO CLIMATIC CONDITIONS ACTING DURING CHILDHOOD AND ADOLESCENCE. Am. J. Hyg., Sect. A, 32:39-43. 1940.

Of 764 cases of cancer occurring between the ages of 20 and 64 in the armed forces of the United States, 284 were tumors of the lips or skin and 480 of the internal

organs. If the patients are divided on the basis of birth site north and south of 40 degrees latitude, skin cancers occurred in 118 northern and 166 southern individuals, while internal tumors were found in 313 northern and 167 southern inhabitants. The greater incidence of superficial neoplasia in southern climates is related to more intense actinic influences. The relatively low incidence of internal cancer in this group is believed by the authors to be directly associated with the more common occurrence of superficial growths and not a chance finding.—M. J. E.

Radiation—Diagnosis and Therapy

DEAN, A. L., and J. BALFOUR. [Memorial Hosp., New York, N. Y.] TREATMENT OF EPITHELIAL TUMORS OF THE BLADDER WITH RADIATION. New York State J. Med., 40:1431-1434. 1940.

The results of irradiation therapy in 50 cases each of papillary carcinoma and infiltrating cancer of the bladder are tabulated. Five years after treatment consisting of roentgen radiation (200 kv., 6,000 r total dose through 6 pelvic portals) combined with electrocoagulation or interstitial implantation of radon seeds, 54% of the patients of the first group and 14% of the second survived.—M. J. E.

del REGATO, J. A. [Academia de Ciencias, Habana, Cuba] LA ROENTGENTERAPIA DE LOS TUMORES DEL SENO MAXILAR, DE LA FARINGE Y LARINGE. [ROENTGEN THERAPY OF TUMORS OF THE MAXILLARY SINUS, PHARYNX, AND LARYNX.] Rev. de med. y cir. Habana, 46:58-65. 1941.

This is a review of the work carried out by the author during 8 years in the Radium Institute of Paris under the guidance of Prof. Coutard. Some of his results were reported at the 3rd International Cancer Congress in 1939. The 5-year cures obtained can be summarized as follows: 4 out of 10 epitheliomas of the upper jaw; 3 out of 18 epitheliomas, 3 out of 13 lympho-epitheliomas, and 8 out of 31 lymphosarcomas of the nasopharynx; 15 out of 37 lymphosarcomas of the tonsil; and 4 out of 11 lymphosarcomas of the base of the tongue. Roentgen therapy of tumors of the hypopharynx, and of the so-called exolaryngeal tumors (mostly epidermoid carcinomas) is very unsatisfactory.—M. D-R.

HUBENY, M. J., and M. MASS. [Cook County Hosp., Chicago, Ill.] ROENTGENOLOGIC ASPECTS OF METASTASES. Radiology, 35:315-321. 1940.

In a series of 1,365 cases of malignant disease studied at autopsy, skeletal metastases were found in 167, an incidence of 12%. This is a considerable increase over previously reported estimates and is attributed to more thorough roentgenographic study and post-mortem examination.—E. A. L.

LEMOS IBAÑEZ, A. [Hosp. Rivadavia, Buenos Aires] ROENTGENTERAPIA DE LOS FIBROMIOMAS UTERINOS. [ROENTGEN THERAPY OF UTERINE FIBROMYOMAS.] Rev. méd-quir. de pat. fem., 17:85. 1941.

The author has treated by this method 450 cases of fibromyoma of the uterus with complete success in 446, and with failure in 4 cases where nodules in the submucosa did not respond to the treatment. Radiation is absolutely contra-indicated in the following cases: (a) pregnancy, (b) presence of co-existing malignant tumors or ovarian cysts, and (c) presence of necrosis or infection in the fibroma itself. Radiation is relatively contra-indicated in the following cases: (a) presence of nodules in the submucosa, (b) presence of pedunculated nodules in the subserosa, (c) large size of the tumors (extending beyond the umbilicus), (d) presence of symptoms of compression which do not call for a surgical operation, and (e) in cases of doubtful diagnosis. Even though radiation can be applied to patients of all ages the author believes that in young women conservative surgery is preferable to radiation. If the latter is adopted it must be applied at doses inducing only temporary castration. Massive radiation is only indicated in the exceptional cases where very severe hemorrhage is present. The author adheres to the view that radiation acts both directly on the tumor and on the ovaries. About 250 references are appended.—M. D-R.

MEDICAL USES OF RADIUM. Summary of reports from experimental research centres for 1939. Edited by the Joint Radiology Committee of the Medical Research Council and the British Empire Cancer Campaign. Brit. J. Radiol., 14:1-10. 1941.

In the Physics Department of the Imperial College of Science and Technology an investigation has been carried out on the production of secondary neutrons as a result of fission of the nuclei of uranium when bombarded by neutrons. It was found that uranium produces secondary neutrons in excess of the number of primary neutrons from the source captured by it. Experiments on the possibility of the production of neutrons in a "chain reaction" are in progress.

At St. Bartholomew's Hospital investigations have been commenced into the use of radio sulfur (S_{35}) as a biological indicator. Using the cyclotron at the Cavendish Laboratory some progress has been made in the isotopic separation of the active sulfur and the detection of its soft radiations.

At the Royal Cancer Hospital work has been continued on the distribution of radiation throughout a volume of tissues when irradiated under therapeutic conditions, and by the use of special instruments it has been found possible to study these distributions in detail. Means have also been devised of finding the mass of tissues raised to given dosage levels so that the "integral dose" throughout the body may be estimated. It has also been possible to compare the energy absorption in the body with that sent into it as gamma or x-ray beams.

At the Radium Beam Therapy Research Centre attempts have been made using a 200 kv. x-ray source to obtain a radiation distribution in tissues which is like that obtained when a teleradium unit is employed. In the course of these investigations new bakelite-graphite materials have been used in the production of ionization chambers which are wave length independent. A mathematical process has been worked out for the computing of volume dosage distributions and the same problem is being attacked experimentally.

The report from the Strangeways Research Laboratory may be conveniently divided into three sections:—

a) The biological effects of radiation. The effects of x-rays on tissue cultures of chick fibroblasts have been

studied at three dose levels, 100 r, 1,000 r and 10,000 r. With doses of the order of 100 r, radiation is followed by temporary diminution of cell division. When an attempt at recovery subsequently occurs, a few degenerate cells appear. With doses of the order of 1,000 r great numbers of abnormal mitotic figures are seen immediately after exposure, indicating an action on cells in the process of division. These abnormalities may be traced after subcultivation. After doses of 10,000 r mitosis is absent and degenerate cells are found soon after the exposure. It has been found that the action of gamma rays on the tadpole eye is essentially similar to that in tissue culture, the same sequence of changes: radiation—diminution in mitosis—subsequent attempt at cell division accompanied by cell degeneration. The work has been continued by a quantitative study of the effect of divided doses in terms of the number of mitotic and degenerate cells seen after exposure. The second irradiation is most effective if delivered at a time when the effects of the first exposure are wearing off.

Similar observations on human malignant cells have been made, the same sequence of events being noted. Quantitative work is being continued with biopsy material.

b) Mechanism of the action of radiation. Using chick fibroblast cultures the effectiveness per röntgen of gamma rays and of x-rays of three wave lengths (0.107, 0.150, and 0.363 A.) has been compared. It was found that the three x-ray wave lengths were equally effective but that the gamma rays were less effective by a factor of the order of two. The results, together with those of neutron experiments, are interpreted in terms of varying density of ions liberated by the different radiations.

The significance of wave length factor in causing a delayed lethal action on chick embryos has also been investigated.

c) The genetical effects of radiation. A study of the production of mutations in *Drosophila* by neutrons and x-rays in the light of the target theory of the action of radiations enables an estimate to be made of the number of genes in the X chromosome.

At the Mount Vernon Hospital investigations have been carried out upon the relative response of the skin of mice to x- and gamma radiation. The ratio of effectiveness for erythema and desquamation was 1.3 and for epilation and exudation 1.6, the x-radiation having greater effect. It appears from experiments on the ionization in chambers containing sulfur that part of the difference is to be attributed to photoelectric emission. Work has also been carried out on the relative effectiveness of neutrons and gamma rays. This effectiveness varies considerably between different biological effects in different materials. Experiments on the inhibition of mitosis appear to show that, whereas gamma rays produce this effect by the cumulative action of a large number of secondary electrons, the inhibition in the case of neutrons may be brought about by a single hydrogen atom recoiling from collision with a neutron.

The results of experiments made at the Institute of Animal Genetics, Edinburgh, with low dosage rates of gamma rays in producing inheritable changes in *Drosophila* show that the production of "gene mutations" and "translocations" is independent of the intensity and timing of the irradiation, and is a function only of the dose. Whereas the frequency of the gene mutation is proportional to the dose, the frequency of the structural change is an "exponential function" changing as about the 1.5 to 2.0 power of the dose.

Work at the Middlesex Hospital throws light on the possibility of stopping the growth of Jensen rat sarcoma by irradiating the surrounding tissues and bed of the tumor while the tumor itself is screened from the radiation. One third of the tumors having only the bed irradiated regressed with a dose which, applied directly, caused 75% to disappear. No effect of varying the size of the irradiated zone around the tumor was observed.

Experiments on the relative effects of the same dose of gamma rays from radium and radon sources on the ovary of rabbits revealed no difference in the effect although the exposure for radon was 11.1 days as opposed to 4.8 days for radium with the same screening.—W. V. M.

Gastrointestinal Tract

BARNES, F. L., and J. P. BARNES. [Houston, Tex.] LIPOMA OF THE COLON. South. Surgeon, 9:853-861. 1940.

A case report of a submucous lipoma of the splenic flexure, clinically and radiologically indistinguishable from carcinoma. The involved area of the bowel was successfully extirpated in a two stage operation.—M. J. E.

BENEDICT, E. B. [Massachusetts General Hosp., Boston, Mass.] CARCINOMA OF THE ESOPHAGUS DEVELOPING IN BENIGN STRICTURE. New England J. Med., 224: 408-412. 1941.

In a series of 40 cases of esophageal carcinoma, the author had 2 cases which followed benign lesions. He lists 31 other cases from the literature. Lye stricture appears to play a definite role in the later development of carcinoma. It was responsible for 16 of the 20 cases in which the exact etiology is mentioned.

The author's 2 cases are presented in detail. One appeared at 58 in a male with a congenital stricture. The second occurred at 35, 34 years after a lye stricture.

Three x-ray photographs and a bibliography of 19 authors are included.—A. M.

BIEREN, R. E. [Sch. of Med., Univ. of Maryland, Baltimore, Md.] CARCINOMA OF THE LOWER INTESTINAL TRACT. Surg., Gynec. & Obst., 72:611-615. 1941.

A study of 254 cases of carcinoma of the large bowel, including 2 of the appendix and 10 of the anus, with a satisfactory follow-up of about 97%. Emphasis is laid on the slow growth and late metastasis of these tumors. Fifty % of the regional node biopsies are inflammatory. In this series of cases pathological grading is of no significance.—H. G. W.

COLL, J., and H. HUMPHREY. [Hartford Hosp., Hartford, Conn.] CANCER OF THE COLON IN TWINS: A CASE REPORT. Connecticut M. J., 5:279. 1941.

The simultaneous occurrence of grade II adenocarcinoma in the colon of presumably homologous twins is reported. —G. De B.

de CHOLNOKY, T. [New York Post-Graduate Hosp., New York, N. Y.] SURGICAL HIGH FREQUENCY CURRENTS

IN CANCER OF RECTUM. Arch. Phys. Therapy, 21:392-396. 1940.

Large inoperable rectal cancers may be coagulated en masse with the electrical current and the coagulated tumor removed with a curette or an electric loop. This procedure readily disposes of necrotic ulcerated masses and produces temporary local alleviation. Satisfactory wound healing is the rule. A case is reported of an inoperable fungating growth treated twice in 7 months. The patient was in good condition and tumor-free 2 years later. The favorable results are demonstrated with photographs.—M. J. E.

de MORAIS, V. [Inst. Portugues para o estudo do cancro, Lisbon] SOBRE CANCRO DO RECTO. [STUDIES ON CANCER OF THE RECTUM.] Arq. de pat., 12:221-449. 1941.

This is a monograph which the specialist has to read in the original. It is divided in 3 parts: I. Etiological factors; clinical and anatomo-pathological types; anatomical evolution; symptoms, diagnosis, and complications; II. surgical and radiological treatment; III. practical application of methods and the factors determining the operability of the neoplasm. The study is amplified by 28 clinical histories from the author's files, and is illustrated by 90 pictures, mostly microphotographs. About 300 references are appended.—M. D-R.

DOUB, H. P., and H. C. JONES. [Henry Ford Hosp., Detroit, Mich.] THE ROENTGENOLOGIC DIAGNOSIS OF TUMORS OF THE SMALL BOWEL. Am. J. Digest. Dis., 7: 149-154. 1941.

Study of 33 cases of histologically verified small bowel tumors.—H. G. W.

FRANKMAN, C. F., and D. H. DRUMMOND. [Wayne Hospital, Greenville, O.] PRIMARY SARCOMA OF THE ILEUM WITH PERFORATION. CASE REPORT. Ohio State M. J., 36:841-842. 1940.

The patient died of an acute peritonitis secondary to a rupture of a fibrosarcoma of the lower ileum.—M. J. E.

GABRIEL, W. B. [St. Mark's Hosp., London] SQUAMOUS-CELL CARCINOMA OF THE ANUS AND ANAL CANAL: AN ANALYSIS OF 55 CASES. Proc. Roy. Soc. Med., 34: 139-157. 1941.

Fifty-five cases of squamous carcinoma of the anus and anal canal were grouped according to their low, medium, or high grade of malignancy on histological grounds. Low grade carcinoma, generally originating at the anal margin, is twice as frequent in males as in females; medium grade carcinoma may arise at the anus or in the anal canal, and is equally distributed between the sexes; high grade carcinoma is much more common in the female sex and is almost limited to the anal canal. Diagnosis and treatment are discussed, and an additional note by R. W. Raven (pp. 157-160) describes the methods and results of treatment in 19 similar cases from the records of the Royal Cancer Hospital (Free).—A. H.

GEYMANN, M. J. [Santa Barbara, Calif.] CARCINOMA OF THE JEJUNUM. Radiology, 36:468-470. 1941.

Report of a case.—E. A. L.

GLENN, F. [New York Hosp., New York, N. Y.] PARTIAL GASTRIC RESECTION FOR CARCINOMA. TWO CASE REPORTS OF FIVE-YEAR SURVIVALS. Ann. Surg., 113:631-636. 1941.

Two successful cases of resection of the stomach are reviewed. Neither patient had metastases in lymph nodes. In the discussion Dr. Pack reviewed hospital statistics of gastric cancer. According to these about 2 patients among 14 resectable cases in 100 admissions for gastric cancer survive 5 years.—A. M.

GLOGGENGIESSER, W. [Path. Inst. der Univ. München, Germany] GENERALISIERTE RETOTHELSARKOMATOSE. [GENERALIZED RETICULUM CELL SARCOMATOSIS.] Virchows Arch. f. path. Anat., 306:506-517. 1940.

A case report. The unusually severe involvement of the intestinal and urogenital tracts and the variable structure of the neoplastic process are stressed. The originally excised growth in the neck, consisting exclusively of undifferentiated cells, is contrasted with the reticular nature of periportal deposits in which production of argentophile fibrils was pronounced.—M. J. E.

HANCHETT, M. [Council Bluffs Clinic, Council Bluffs, Iowa] EARLY DIAGNOSIS OF CARCINOMA OF THE COLON. J. Iowa M. Soc., 30:526-528. 1940.

Cancer of the colon in its earliest recognizable stage produces only vague persistent intestinal symptoms. These are frequently disregarded by the patient and physician and valuable time is lost. Rectal examination, proctosigmoidoscopy and thorough roentgen studies are capable of revealing small tumors. In consequence of competent early diagnosis operative intervention may be performed with a distinct possibility of effecting a permanent cure.—M. J. E.

HOGEBOOM, G. W. [Springfield, Mo.] CARCINOMA OF THE SIGMOID AND THE ADVANTAGES OF THE DEVINE COLOSTOMY. J. Missouri M. A., 38:20-21. 1941.

Two cases are recorded in which successful resection of cancer of the lower colon was performed according to the technic of Devine (preliminary colostomy with defunctionalizing of the distal colon followed by resection of the tumor and end to end anastomosis).—M. J. E.

HUNT, E. L., and G. D. KANEB. [Worcester City Hosp., Worcester, Mass.] PRIMARY ADENOCARCINOMA OF THE JEJUNUM. New England J. Med., 224:353-357. 1941.

The authors present 3 cases of adenocarcinoma of the jejunum. Two are alive and well 12 and 7½ years after resection and end to end anastomosis. The third died 2 years and 4 months after operation with extensive metastases to the femur.—A. M.

HUNT, V. C. [Los Angeles, Calif.] SURGICAL MANAGEMENT OF CARCINOMA OF COLON. California & West. Med., 35:258-261. 1940.

Diagnostic methods, preoperative preparation of patients, and the various types of resection of the colon performed either in one stage or in two stages are outlined.—M. J. E.

JANKELSON, I. R., and C. W. McCLURE. [Boston City Hosp., Boston, Mass.] LIMITATIONS OF GASTROSCOPY. New England J. Med., 224:64-67. 1941.

The article emphasizes that gastroscopy ought not to supersede but complement roentgen ray examination of the stomach. The authors discuss the chief difficulties they have had with 800 gastroscopies in a 3½ year period. Small gastric lesions, particularly about the lesser curvature of the cardia and upper third of the stomach, are readily missed. The differentiation between ulcerating cancer and benign ulcer is subject to error and requires repeated examination. Gastric mucosal changes may be secondary to disease elsewhere and a careful clinical

history is necessary for proper diagnosis. The discovery of marginal ulcers or the evaluation of functioning stomas is difficult. A bibliography of 18 papers is given.—A. M.

KERR, J. G. [Baylor Univ. Coll. of Med., Dallas, Tex.] SQUAMOUS CELL CARCINOMA OF THE ANORECTAL REGION. Tex. State J. Med., 36:548-551. 1940.

Of 56 patients on whom a significant estimation of the course of disease was obtainable following operative removal of anorectal cancer, 16 (28.5%) survived 5 years after treatment. If in this group are considered only the 34 patients with operable tumors treated radically, 14 (41%) survived after 5 years.—M. J. E.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27042. New England J. Med., 224:168-170. 1941.

A case of a benign lipoma of the hepatic flexure of the colon is reported.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27061. New England J. Med., 224:248-250. 1941.

A case of neurogenic fibrosarcoma of the stomach is discussed.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27091. New England J. Med., 224:384-386. 1941.

A case of adenocarcinoma of the transverse colon is presented. Urinary symptoms caused by invasion of the bladder preceded bowel symptoms by 9 months.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27092. New England J. Med., 224:386-388. 1941.

A case of low grade papillary carcinoma, primary in the terminal portion of the duodenum, is presented.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27111. New England J. Med., 224:467-473. 1941.

A case of widespread reticulum cell sarcoma of uncertain origin involving the stomach, gastrocolic omentum, retroperitoneal tissues, mediastinum, pleura, right lung, etc., is presented.—A. M.

MAYO, C. W. [Mayo Clinic, Rochester, Minn.] MALIGNANCY OF THE SMALL INTESTINE. West. J. Surg., 48: 403-407. 1940.

The clinical aspects of primary cancer of the small bowel are reviewed on the basis of 108 cases observed between 1907 and 1939. Surgery is the accepted treatment, but radical resection was possible only in 42.4% of the cases; palliative measures were resorted to in 41.3%, and in 16.3% intervention was limited to exploration. Of 64 patients in whom a radical resection of the tumor was performed 5 or more years previously, 8 survived this period, but in this group 5 died of unstated causes between 5 and 18 years. Of interest histologically is the fact that all 4 tumors observed in a persistent Meckel's diverticulum were leiomyosarcoma, while this form constituted only 10% of the total tumors in the small intestine.—M. J. E.

MAYO, C. W., and W. R. LOVELACE. [Mayo Clinic, Rochester, Minn.] MALIGNANT LESIONS OF THE CECUM AND ASCENDING COLON. Surg., Gynec. & Obst., 72:698-706. 1941.

A statistical study of 885 cases of malignant lesions of the cecum, ascending colon, and hepatic flexure, which constitute 30% of the lesions in the abdominal colon, and 12% of the malignant lesions of the entire colon and anal canal. Resection with a view to cure was performed in 67% of the cases in this series which is higher than in malignancies elsewhere in the large intestine. The net hospital mortality rate was 23.5% and the 5-year survival rate was 57%. Grading of the neoplasms by the method of Broders is of prognostic value.—H. G. W.

MUFSON, S., and E. A. HOROWITZ. [Beth Israel Hosp., New York, N. Y.] INTUSSUSCEPTION OF THE JEJUNUM DUE TO CARCINOID TUMOR. New England J. Med., 224: 602-605. 1941.

In adults, 40% of intussusception cases are due to tumors, most of which are benign. Of 237 carcinoid tumors in the literature only 3 resulted in intussusception. All 3 were in the ileum. In the case reported, intussusception resulted from a mushroom-shaped carcinoid in the lumen of the jejunum. The tumor was successfully resected.—A. M.

NESSLER, A. B. [Finley Hosp., Dubuque, Iowa] CARCINOMA OF STOMACH ASSOCIATED WITH PERNICIOUS ANEMIA. J. Iowa M. Soc., 30:410-411. 1940.

A report with autopsy record of a case of adenocarcinoma of the stomach in a patient who suffered from pernicious anemia 4 years previously. A complete clinical remission had been obtained with liver therapy.—M. J. E.

OCHSNER, A., and M. DeBAKEY. [Sch. of Med., Tulane Univ., New Orleans, La.] SURGICAL ASPECTS OF CARCINOMA OF THE ESOPHAGUS. J. Thoracic Surg., 10:401-445. 1941.

Review of literature, with 295 references, and report of 4 operated cases, with one patient living over $1\frac{1}{2}$ years after the operation. Of the total number of 195 cases in which resection was performed there was an operative mortality of 71.8%. This paper is followed by a pertinent paper on experimental esophagastrostomy, and a discussion.—H. G. W.

PLATT, O. R., G. F. WALTEMATH, and J. H. MILLHOUSE. [North Platte, Neb.] EXOGASTRIC PEDUNCULATED MYOSARCOMA OF STOMACH. Nebraska M. J., 25: 336-337. 1940.

The tumor in this patient was associated with profound anemia and weakness and, although palpable, gave rise to no symptoms directly referable to the stomach. It was successfully extirpated.—M. J. E.

PRESSLY, T. A. [San Antonio, Tex.] CARCINOMA OF THE DESCENDING COLON. South. Surgeon, 9:836-842. 1940.

A report of 2 cases in patients aged 74 and 76. The tumors were extirpated radically and 1 patient survived in good condition. The second died after 2 months, of a complicating cellulitis of the neck.—M. J. E.

STEIN, J. J. [Edward Hines, Jr. Hosp., Hines, Ill.] METASTASIS TO BONE FROM CARCINOMA OF THE GASTROINTESTINAL TRACT. Radiology, 35:486-488. 1940.

This is a study of bone metastases based upon the findings in 1,005 cases of carcinoma of the gastrointestinal tract. The most common site for the primary carcinoma was the stomach, followed in order by the rectum, colon, and esophagus. The incidence of bone metastases was 2.65% for stomach, 5.93% for rectum, 0.93% for colon, and 2.94% for esophagus. The presence of carcinoma and incidence of metastases were less for other portions of the tract. These findings are compared with other reports

occurring in the literature. The incidence of metastasis is higher than generally thought. The majority of the metastatic lesions are of the osteoclastic type.—E. A. L.

STOLL, J. B. [Clay Center, Kan.] CARCINOMA OF CECUM SIMULATING APPENDICITIS. J. Kansas M. Soc., 41:468-469. 1940.

A report of a case in which successful excision of the tumor was performed.—M. J. E.

THORLAKSON, P. H. T., and S. MELTZER. [Winnipeg, Canada] INTRACTABLE GASTRIC ULCER WITH FINAL MALIGNANT CHANGE ASSOCIATED WITH A BENIGN TUMOR OF THE BRAIN. Ann. Surg., 113:521-527. 1941.

The authors emphasize the importance of the neurogenic theory of origin of peptic ulcer and quote from the literature to demonstrate the frequent concomitance of intracranial lesions and peptic ulceration. The case reported had a subtemporal decompression for an intracerebral tumor. Ulcer symptoms were present at that time and responded to diet but later necessitated three operations: excision of the ulcer, gastroenterostomy, and feeding jejunostomy. The patient succumbed almost 16 years after the onset of her decompression. Autopsy showed a fibrillary astrocytoma of the right frontal lobe compressing the corpus callosum and third ventricle. The gastric ulcer showed "beginning malignant change." A diagram schematically presents the possible action of the brain tumor on the gastric lesion. Three photographs are shown. There is a bibliography of 20 papers.—A. M.

Conference on Gastric Cancer, National Advisory Cancer Council, October 11 and 12, 1940

BRUNSCHWIG, A., R. L. SCHMITZ, and R. RASMUSSEN. [Univ. of Chicago, Chicago, Ill.] EXPERIMENTAL OBSERVATIONS ON ACHLORHYDRIA OF GASTRIC CANCER. J. Nat. Cancer Inst., 1:481-488. 1941.

Dogs were prepared with gastric pouches and were desensitized to the injections of extracts of human tissues. They were then fed, and after the flow of gastric juice in the pouch was measured as a control value, they were injected intravenously with either an extract of cancerous or control human stomachs or the secretions from such stomachs. It was found that the extracts and juices from achlorhydric (cancerous) stomachs in the great majority of instances had a pronounced depressant effect upon the secretion in the pouch while only a few samples of control extract produced a similar effect. Extracts, furthermore, prepared from the achlorhydric stomachs of patients with pernicious anemia were equally as effective in depressing gastric secretion as were the cancerous stomachs. The depressant factor is nondializable and heat labile. It is believed not to be enterogastrone.

The paper is discussed by Dr. A. C. Ivy.—L. L. W.

COLLINS, S. D., M. GOVER, and H. F. DORN. [National Institute of Health, U. S. Public Health Service] TREND AND GEOGRAPHIC VARIATION IN CANCER MORTALITY AND PREVALENCE, WITH SPECIAL REFERENCE TO GASTRIC CANCER. J. Nat. Cancer Inst., 1:425-450. 1941.

A summary of statistical data reveals that the recorded mortality from all cancer in the United States has increased steadily since 1900. The increase has affected males more than females. However, since 1920 in females and since 1922 in males, there has been a slight decrease in the death rate from cancer of the stomach and liver. The decrease has been more marked in women. The highest recorded death rates from cancer of the stomach and duodenum in the years 1930-32 occurred in the northern states from the Atlantic to the Pacific. The southern states had uniformly low mortality rates in this respect. On the other hand, mortality rates for skin cancer are much higher in the South than in the North.

From census figures of current cancer cases in the white populations of 12 large cities in the North, there were 2.3 cases for every annual death as compared with 4.0 per death in the South and 2.8 in the West. There were 24 cases of skin cancer under treatment for each annual death in all areas surveyed, but only 1.4 digestive tract cases.

Among white males, cancer of the digestive system made up 34% of the cancer cases and 54% of the deaths; in white females cancer in this site accounted for 20% of the cases and 40% of the deaths.

Resident cases of all forms of cancer in the surveyed areas amounted in the North to 282 per 100,000 white population, 386 in the South, and 425 in the West. The South showed a lower cancer death rate but a higher rate of cases under treatment than the North. Cancer of the stomach, intestine, and rectum was more prevalent in the white population in the cities of the North than in those of the South.

There is discussion by Dr. C. C. Little, Dr. M. T. Macklin, Dr. Walter Alvarez, Dr. Albert Tannenbaum, Dr. E. V. Cowdry, and Dr. Joseph O'Brien.—L. L. W.

KLEIN, A. J., and W. L. PALMER. [Univ. of Chicago, Chicago, Ill.] EXPERIMENTAL GASTRIC CARCINOMA: A CRITICAL REVIEW WITH COMMENTS ON THE CRITERIA OF INDUCED MALIGNANCY. J. Nat. Cancer Inst., 1:558-584. 1941.

This paper is reprinted with additions, from Arch. Path., **29**:814-844. 1940. It is a comprehensive review of the subject of experimental gastric cancer with a bibliography of some 60 titles. The criteria of induced malignancy are summarized as follows:

Proof of malignancy is not given by the histologic appearance of a tumor. It is given by (1) the ability of the tumor to proliferate independently as metastases; (2) the ability to invade progressively and destructively neighboring tissues; (3) the irreversibility of these activities which must be shown to continue in the absence of the extrinsic factor initially held responsible for the cellular changes; and (4) it must be shown that a given malignant change occurs with sufficient regularity to establish a causal relationship of the experimental procedure to the cancer.—L. L. W.

REID, M. R. [Univ. of Cincinnati, Coll. of Med., Cincinnati, Ohio] THE USE OF CLINICAL MATERIAL FOR THE INVESTIGATION OF GASTRIC ULCERS. J. Nat. Cancer Inst., 1::523-537. 1941.

The author describes the organization and work of the gastric research laboratory associated with the gastric clinic at the University of Cincinnati. He concludes that the coordinated efforts of the clinician, gastroscopist, roentgenologist, pathologist, and other specialists have resulted in concrete advances in the knowledge of gastric disease and how to treat it. He stresses the importance

of carefully assaying the nutritional state of gastric cases before operation. Finally, he cautions against complete dependence upon laboratory tests. They should serve to aid sound clinical judgment and not overrule it.

The paper is discussed by Drs. C. P. Rhoads, J. J. Morton, A. R. Dochez, and W. Alvarez. Dr. Alvarez' discussion brings out many noteworthy clinical facts based on the statistical data from the very large series of gastric cases (10,890) studied at the Mayo clinic by Dr. Walters and Dr. Lewis.—L. L. W.

RHOADS, C. P. [Memorial Hosp., New York, N. Y.] GASTRIC CANCER AS A SEQUEL TO GASTRITIS, PARTICULARLY THE GASTRITIS OF PERNICIOUS ANEMIA. J. Nat. Cancer Inst., 1:511-522. 1941.

The evidence supporting the assumption that certain gastric cancers develop in a gastric mucosa, the seat of a concomitant or precedent chronic gastritis, is marshalled from the literature. The chief points emphasized are the chronicity of gastric symptoms in some patients developing gastric neoplasms, the frequency with which gastritis is found in cancerous stomachs removed at operation or autopsy, the frequent association of gastric polyposis or carcinoma as a sequel of pernicious anemia, and an hereditary tendency in some families to develop one or the other of these disorders.

If chronic gastritis is really a precancerous condition in some cases, as it would seem from the above, then therapeutic measures aimed at clearing up these lesions would constitute a logical prophylaxis. For the gastritis of tropical sprue and that of pernicious anemia, dietary supplements are effective.

The paper is discussed by Drs. L. Schiff and R. Schindler. —L. L. W.

SCHINDLER, R. [Univ. of Chicago, Chicago, Ill.] EARLY DIAGNOSIS OF CANCER OF THE STOMACH; GASTROSCOPY AND GASTRIC BIOPSIES, GASTROPHOTOGRAPHY, AND X-RAYS. J. Nat. Cancer Inst., 1:451-480. 1941.

The author reviews the technics involved in the early diagnosis of gastric cancer. From his own experience, he concludes that almost every gastric carcinoma no matter how small can be diagnosed by combining improved x-ray visualization with gastroscopy. Not only is he able to diagnose cancerous growths in the stomach but he has been able to grade them macroscopically—a distinct aid in surgical prognosis. Further, in a majority of cases he has been successful in distinguishing benign from malignant ulcers of the stomach and in recognizing alleged precancerous conditions including gastric polypi and chronic atrophic gastritis.

Gastrophotography may be used to record the gastroscopic findings. Gastroscopy is apparently a safe procedure and is technically easy in trained hands. Only one fatality has been reported in 22,351 gastroscopies, and this was not conclusively attributable to the procedure.

In light of the above evidence, Dr. Schindler feels that failure to advise prompt x-ray examination and gastroscopy in patients with minor digestive symptoms is inexcusable.

The paper is well illustrated. There is discussion by Dr. James Ewing, Dr. Max Zinninger, and Dr. Gordon McNeer.—L. L. W.

STEWART, H. L. [Nat. Cancer Inst., Bethesda, Md.] HYPERPLASTIC AND NEOPLASTIC LESIONS OF THE STOMACH IN MICE. J. Nat. Cancer Inst., 1:489-509. 1941.

Earlier observations by the author and his associates on hyperplastic and neoplastic lesions of the stomach are reviewed. The spontaneous adenomatous growths occurring in strain I mice are described in detail. These lesions appear in the pyloric or glandular portion of the stomach and as far as is known are not malignant. The tendency to develop the lesion is apparently inherited as a recessive characteristic. No relation to vitamin deficiency has been found.

In 8 of 30 strain A male mice the author succeeded in producing 4 squamous cell carcinomas and 4 papillomas of the forestomach by injecting a solution containing 10 mgm. methylcholanthrene directly into the wall of the stomach. The 4 carcinomas, although keratinizing, were thought to be definitely malignant. Experiments are also described in which squamous cell carcinomas of the forestomach have been induced by the oral administration of 20-methylcholanthrene in aqueous mineral oil emulsion to A strain or A backcross mice. Certain of these tumors showed metastases and other criteria of true malignancy.

The paper is discussed by Drs. W. U. Gardner, A. W. Oughterson, E. Lorenz, M. J. Shear, and F. Hollander.—L. L. W.

VOEGTLIN, C. [Nat. Cancer Inst., Bethesda, Md.] A PROGRAM FOR THE STUDY OF CANCER OF THE STOMACH. J. Nat. Cancer Inst., 1:539-558. 1941.

The approaches to the study of gastric cancer presented at the Conference on Gastric Cancer held at the National Institute of Health, Bethesda, Maryland on October 11 and 12, 1940, are briefly reviewed. From these researches, definite problems for future work on gastric cancer are proposed as follows:—

1. Statistical studies on mortality, incidence, geographic distribution.
2. Epidemiological studies.
3. Clinical studies on causation.
   a. Relation of atrophic gastritis to gastric cancer.
   b. Relation of pernicious anemia to gastric cancer.
   c. Further studies on achlorhydria.
4. Laboratory studies on causation.
   a. Production and study of gastric adenocarcinoma by carcinogenic hydrocarbons.
   b. Production of nonmalignant and malignant gastric lesions by dietary factors.
5. Studies on improvements of diagnostic procedures.
   a. Training of competent radiologists and gastroscopists.
   b. Further work on the gastroscope as a diagnostic instrument.
   c. Central diagnostic bureaus.
6. Organization of a coordinated clinical gastric cancer service.
7. Education of the laity.

A general discussion of problems in gastric cancer is appended. Those participating are Drs. S. Warren, G. Holmes, J. J. Morton, A. W. Oughterson, M. C. Winternitz, M. T. Macklin, A. R. Dochez, C. C. Little, W. Alvarez, A. Brunschwig, and S. Bayne-Jones.—L. L. W.